The Journal of Parasitology

Volume XX MARCH, 1934 Number 3

SOME CELLULAR BASES FOR IMMUNE REACTIONS IN PARASITIC INFECTIONS[1]

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The rapidly increasing interest in problems of parasitic immunity during the last few years has led me to hope that my present subject will touch upon the research interests of a number of parasitologists.

In analyzing all cases of acquired and many cases of natural immunity, it is generally conceded that no matter what the immediate mechanism may be, ultimately, the immunity rests on certain cellular activities. In developing this subject it is not my purpose nor is it within my competency to digest our knowledge of the cellular reactions to parasites or even our somewhat limited knowledge of the cellular reactions known to assist in defense reactions. Rather, I wish to select certain examples, as illustrative of the evidence that is accumulating, which tend to show that defense of the vertebrate against foreign bodies or invading agents, whether the latter be vegetable or animal in nature, predominantly involves the cells of one great system. These are the connective tissue cells which embryologically are derived from the mesenchyme. Provided this system be at the base of all immune reactions, it follows logically that there should be a unified concept of immune reactions rather than special systems for each group of invaders. In developing this concept I shall outline the origin and function of the cells in local inflammation in normal animals produced by sterile materials and bacteria, the essential local character of general immunity, and the cellular basis for immune reactions to certain bacterial, protozoal and metazoal parasites.

At the outset it should be borne in mind that the connective tissue is a widely distributed system possessing manifold normal functions. It consists of the blood and lymph, blood-forming (reticular), loose connective and adipose tissues, cartilage and bone, and its normal functions vary from respiration, intermediate metabolism and storage to mechanical support. The defense reactions, however, are largely limited to the blood

[1] Address of the Retiring President, American Society of Parasitologists, December 30, 1933, Boston. The author is greatly indebted for criticism from his colleagues, Prof. Paul R. Cannon and Prof. William Bloom.

and lymph, reticular and loose connective tissues, and are probably secondary to or accentuations of normal functions. It seems not unlikely that the cells of these tissues are active in defense because of certain characteristics, chief among which are: their ubiquity, their retention of phagocytic powers, their ability to elaborate digestive enzymes and their potentialities for development, and hence, regeneration and repair. Such physiological properties are possessed to a lesser degree by all other body cells and can often be evoked by strong enough stimuli, but are not often evident because the stimuli are generally effectively removed by the blood and reticular and connective tissues.

CELLULAR REACTIONS IN NORMAL ANIMALS (NATURAL IMMUNITY)

A local inflammatory response follows the local introduction of a foreign stimulant into the connective tissue of normal animals and has been found to involve the successive interplay of various cells. Credit should be given to Metchnikoff for insisting upon the essential rôle of the mesenchymal cells in inflammation and to Cohnheim, Ziegler, Marchand and Maximow among others for studying the histogenesis of the local inflammatory reactions. Although the general function of these cells is agreed upon, their origin has given rise to as much controversy as any question in biology. The cells involved may be classified in the order in which they make their appearance, as follows:

1. *The polymorphonuclear leucocytes*[2] (*neutrophiles in man*). Under normal physiological conditions the polymorphonuclear leucocytes are formed in the bone marrow from the myeloid tissue[3] and are mainly limited to the blood vessels, but as one of the immediate reactions in local inflammation they migrate from the vessels into the site of inflammation. Their number, activity and length of stay depend upon the severity and chemical nature of the inflammatory stimulus and whether it is sterile or septic. When sterile, they soon disappear, but under sepsis they combat the invading organisms in many visible ways: by active phagocytosis, by digestion, by the secretion of bactericidal and proteolytic ferments, etc. They represent the most easily mobilized cells and although very important as a first line of defense are potentially limited since they are recruited from the blood stream, do not arise or multiply *in situ* or develop into other cells of the area.[4] They are short-lived and generally disintegrate within a comparatively few days.

[2] Termed microphages by Metchnikoff, because they phagocytose small particles such as bacteria, and special leucocytes by Maximow, because they vary characteristically with different species.

[3] For a criticism of the view of von Möllendorff and his students that polymorphonuclear leucocytes, eosinophiles and macrophages arise from the fibroblasts of the loose connective tissue, see Maximow (1927) and Fischer-Wasels (1928).

[4] For a criticism of the suggestion by Lewis (1926) that polymorphonuclears can develop into macrophages, see Maximow (1927).

2. *Mononuclear exudate cells (polyblasts).* This series of cells is characterized by varied developmental and phagocytic potentialities. The smallest are indistinguishable from the small lymphocytes of the blood and lymphatic organs and arise chiefly by migration from the blood stream. The slightly larger monocytoid cells arise partly by the migration of monocytes from the blood and partly by hypertrophy of the lymphocytes. The macrophages arise by division of preëxisting macrophages, by the direct transformation of the outstretched fixed perivascular cells of the area (which possess mesenchymal potencies) and by transformation of the lymphoid-monocytoid series. Hence, at the site of inflammation, a progressive gradation from small lymphocytes to intermediate cells with monocytoid nuclei, to large cells with macrophage nuclei comprise the mononuclear exudate cells, *i.e.,* the polyblasts. Of these cells the small lymphocyte is not phagocytic, the monocytoid cell is phagocytic to a small extent, and the macrophage is highly phagocytic.

The macrophages[5] are undoubtedly the most important in local defense. They appear later than the polymorphonuclear leucocytes and rapidly increase as the former finish their work. They actively phagocytose and digest certain invading organisms, clear up cellular and other debris and elaborate antibodies which aid in phagocytosis. When large foreign bodies exist, they may fuse to form foreign body giant cells, when microorganisms are indigestible, they may form giant cells such as the epithelioid cell of the tubercle, or when large areas are necrotic they may surround the area, become transformed into fibroblasts and effectively wall it off.

3. *The fibroblasts.* Fibroblasts are a prominent constituent of normal loose connective tissue. They react slowly and probably play an active part only in the later stages of local inflammation when their activity is largely limited to reparative processes such as the regeneration of new connective tissue, replacement of cavities, and the walling off, *i.e.,* the isolation of foreign bodies or old foci. The fibroblasts in this late stage have a multiple origin. Many arise from preëxisting fibroblasts *in situ* or from the true endothelium of the small blood vessels. Many arise from preëxisting macrophages *in situ* or from migratory blood lymphocytes or monocytes.[6]

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[5] I have used the term macrophage or macrophage system because of its functional, viz., phagocytic, implication, as first proposed by Metchnikoff. The macrophage system is essentially the same as the reticulo-endothelial system of Aschoff and Landau (1913) and includes in general: reticular cells (fixed macrophages) of the pulp and sinuses of the spleen and other lymphatic organs, phagocytes lining the sinuses in the liver (Kupffer cells) and bone marrow, and histiocytes (clasmatocytes, wandering macrophages) of the loose connective tissue.

[6] Some evidence indicates that under continued stimulation the fibroblast can become a macrophage.

4. *Eosinophiles (and basophiles?).* The eosinophiles are of myeloid ancestry and are closely related to the polymorphonuclear leucocytes. Ordinarily, the eosinophiles are formed in the bone marrow and are limited to the blood stream until certain local inflammatory stimuli cause them to migrate into the tissues. There are some exceptions to this.[7] Thus, they are formed in scattered granulopoietic foci in the lamina propria of the intestinal mucosa. They seem to play an important part in chronic inflammation, especially in the resorption of protein disintegration products and are particularly prominent after the body has become sensitized to the particular protein. As a rule, therefore, they come into play during late stages of the defense reaction, but like their relatives, the polymorphonuclears, are limited by their lack of developmental potentialities.[8]

Ordinarily when the stimulant is generally distributed over a large part of the body, the reaction is designated as a general defense reaction, but as a matter of fact such a distribution ordinarily signifies that it is in the blood stream and the ensuing reaction is essentially different only in degree and generally is localized in certain organs. The same types of cells are involved, but the extent to which they are involved depends upon the nature of the foreign material or infectious agent. Here again, the polymorphonuclears are often mobilized first, but the lymphoid-monocytoid-macrophage series shows the most pronounced histological changes and the macrophages seem to bear the brunt of the activity. The essential repair work of the fibroblasts is often not in evidence at all. The mesenchymal cells of the liver, spleen, bone marrow, etc., show the most extensive histological changes because such sites are not only advantageously placed but possess sluggish circulation where specific cells can come into contact with the stimulant. In a very real sense, these cases represent local reactions in strategically placed organs.

CELLULAR REACTIONS IN IMMUNE ANIMALS (ACQUIRED IMMUNITY)

The same cells which react to invading organisms or non-infectious antigens in the normal animal also react in the immunized animal with the difference that the macrophages generally take an even more prominent rôle, are far more numerous and individually are far more reactive. The greater reactivity of the macrophage in the immune animal is associated with the intermediary action of antibodies in some and possibly in all cases. Such antibodies also probably give to acquired immunity its

[7] Their apparently constant occurrence in normal loose connective tissue in the rat, mouse and guinea pig may be due to some chronic infection or parenteral intoxication, according to Weidenreich, Maximow and others.

[8] For a criticism of von Möllendorff's contention that eosinophiles can develop into polymorphonuclear leucocytes and later into nongranular leucocytes, see Maximow (1927).

specific character which is lacking in natural immunity. Furthermore, just as in natural immunity, an acquired general immunity is a local immunity of certain organs to which the invading organism has access or for which it seems to have a predilection.

Modern workers in local immunity owe a great debt to Besredka for stressing the elective specificity and limited portal of entry of invading organisms with the correlated demonstration that successful immunization against a particular organism involves the specific portal of entry and the specific susceptible organ. He recognized the essential activity of the cells, but excluded antibodies even to the extent of defining local immunity as "immunity without the obligatory participation of antibodies." A much better definition of local immunity as "due to a locally superior mechanism for the disposal of a particular microörganism" has been given by Gay. He and his co-workers (see Gay, 1931) have demonstrated the greatly enhanced action of the macrophages against bacteria in locally immune as compared to normal animals by direct microscopical observations and indirect experimental procedures. To explain the macrophage activity in local acquired immunity, Gay (1931) believes either that the macrophages secrete opsonizing or tropinizing antibodies which in turn render the invaders more easily phagocytosable or that the macrophages are themselves changed (attuned, according to the terminology of certain German authors) so that they are more active towards certain specific forms. As pointed out by Cannon and Pacheco (1930), however, when investigators have attempted to exclude antibodies they have generally meant *serum* antibodies and have not given adequate consideration to the local production of opsonins which may be effective locally, but ineffective after diffusing into the blood stream where they are greatly diluted.

Many of these points are illustrated by the work of Cannon and Pacheco (1930) who compared the reactions to *Staphylococcus aureus* when injected intracutaneously into normal guinea pigs on the one hand and into locally immunized guinea pigs on the other. In normal animals the inflammatory response predominantly involved the polymorphonuclear leucocytes which although they actively phagocytosed the bacteria did not prevent their dissemination throughout the subcutaneous tissues. In immunized animals there was a marked thickening of the subreticular layer of the subcutis due largely to increased numbers of macrophages. When these animals were injected with the bacteria there was a local agglutination and intense phagocytosis by the macrophages which prevented spread of the infection. It seems not improbable from this and other work of Cannon and his collaborators that both the agglutination and phagocytosis are specific and mediated by antibodies locally produced.

NATURAL AND ACQUIRED IMMUNITY IN PARASITIC INFECTIONS

Recent investigations at the University of Chicago by Prof. Cannon and the speaker on the cellular basis of immunity to both avian and simian malaria (see Cannon and Taliaferro, 1931, and Taliaferro, 1932) illustrate the direct rôle of the macrophage and the stimulation of the entire lymphocyte-macrophage system in a parasitic immunity. Thus, the disappearance of *Plasmodium cathemerium* and *P. brasilianum* in all stages of the infection can be directly correlated with the activity of the differentiated macrophages of the spleen and liver and to a less extent of the bone marrow. During the acute rise of the initial attack there is a large constant mortality of the parasites which represents a natural immunity and which is correlated with a comparatively sluggish phagocytic activity of the macrophages of these organs. The termination of the initial attack, the subsequent low grade infection and subsequent immunity to superinfection are associated with an acquired immunity and involve a greatly heightened activity of the macrophages of the same organs with a consequent greater mortality of the parasites. Although both natural and acquired immunity involve the macrophages, the first is nonspecific and comparatively ineffective, the latter highly specific[9] and very effective. Furthermore, there are many reasons for believing that the greater and specific activity of the macrophages of the immune host is due to an opsonizing antibody (see Taliaferro, 1932).

As the malarial infection progresses and concomitantly with the activation of the macrophages, there is an activation of the lymphatic system as evidenced by increased mitosis of all three types of lymphocytes and the reticular cells, particularly of the spleen.[10] To many, the lymphocyte is a cell of unknown function and a lymphocytic activation is considered to be of no proven rôle in defense. In Maximow's original experiments on local inflammation, on the contrary, the blood lymphocyte not only migrates to the site but apparently metamorphoses into a monocytoid cell which later may become a macrophage. The possibility of this sequence is strengthened by the same development in tissue cultures (see Bloom, 1932). In sections of spleen conclusive evidence is impossible, but the most probable explanation of our findings in malaria is that the lymphocytes constitute a mesenchymal reserve from which macrophages arise under intense malarial stimulation.

Malaria is a generalized infection to the extent that it is distributed throughout the blood stream, and yet, practically, only the macrophages

[9] See reports of the specificity of immunity in avian malaria by Gingrich (1932), in simian malaria by Mulligan and Sinton (1933) and review of human malaria by Thomson (1933).

[10] A similar lymphoid activity is observable in many infections not due to parasites and in many cases of immunization with non-infective antigens.

of the spleen, liver and bone marrow are involved in the defense. This is probably a matter of location and opportunity. In other words, the circulation in these sites is more or less sluggish and the infected erythrocytes come in contact with the macrophages. They do not come into prolonged contact with the macrophages of other organs. Thus, the macrophages (septal cells) of the lungs are highly active for material introduced into the bronchioles, but can rarely remove material from the blood stream unless there is a stasis. The macrophages of the omentum and the mesenteric lmyph nodes can remove material from the peritoneal cavity, but again have little opportunity for removing material of hematogenous origin. In a real sense, then, the immune reactions in malaria are local responses in strategically placed organs.

In fact, as previously stated, it is probable that most of our so-called general immunities are local immunities. The most general immune reaction in the parasites of which I am aware is the case of *Leishmania donovani* where the reaction is signally ineffective because the macrophages ingest the parasites but are unable to digest them (see review in Linton, 1929). It seems probable that at first the same local reaction occurs as in malaria, but that as it proves ineffective and as the parasites increase in numbers and become distributed, the macrophage response becomes more and more generalized.

The direct histological evidence of the rôle of the macrophages, just presented, has been supplemented by indirect methods, such as by splenectomy and the so-called blockading techniques. Since the spleen is fundamentally a large lymph node and represents the greatest single accumulation of lymphatic tissue and macrophages in the body, its removal should deprive the body of a considerable fraction of the macrophage system and thereby lower the body's defense. The fact that clear cut results have not always been obtained is probably due to the variability in the spleen-weight: body-weight ratio in different species of animals and to the rapid compensatory hyperplasia in other lymphatic organs of the body after splenectomy (see Krumbhaar, 1926). In malaria of monkeys and several other protozoan infections splenectomy manifestly lowers the acquired resistance and initiates relapses.[11] That the spleen possesses peculiar properties in addition to its content of lymphatic and reticular cells, as maintained by some investigators, seems unlikely in view of the ease with which a large part of the loss sustained through splenectomy is made good by the body.

Inasmuch as various colloidal dyes, particulate matter, foreign cells and serum, when they can be traced, are also taken up by the macrophages, their introduction should also depress immune reactions by decreasing the effective macrophage function. Such procedures have been

[11] See reviews in Linton (1929), Jungeblut (1930) and Taliaferro (1929).

shown to lower the immune responses in many conditions (see Jungeblut, 1930), but their success probably depends upon a fine precision in technique because inadequate dosage, on the one hand, may actually stimulate the macrophages whereas anything approaching complete blockade, on the other hand, may kill the animal by depriving it of too many normal physiologic functions.

The phagocytic character of the immunity in malaria is self-evident, but indirect evidence in some immune reactions not leading to phagocytosis points to the macrophage as the locus of antibody-formation. A good example is furnished by *Trypanosoma lewisi* which reproduces in the rat by asexual fission during the first 10 days of infection and thereafter remains in the blood without reproducing. In 1924 the speaker showed that this inhibition of reproduction was associated with a specific antibody which is passively transferable to normal rats and which inhibits reproduction of the parasites without killing them. Subsequent work by Regendanz and Kikuth, by Marmorston-Gottesman, Perla and Vorzimer and by the speaker in collaboration with Cannon and Goodloe showed that the formation of this antibody was greatly lessened by splenectomy, India ink blockade, pregnancy and Bartonella and paratyphoid infections and was pronounced when two or more acted simultaneously. (See review in Taliaferro, 1932b.)

Moreover, according to work of mine on this same infection, one or more trypanocidal antibodies are formed which kill the trypanosomes by extracellular lysis or opsonization. These, likewise, are affected by splenectomy and India ink blockade. Similarly, Amako (1930) has shown that the spleen and to a slightly less extent the liver are the chief sites of formation of antitrypanosome agglutinating antibodies produced in rabbits with a trypanosomal vaccine.

The past five years has seen a brilliant series of investigations on acquired immunity in helminth and other metazoan parasites. In none of these, as far as I am aware, has the essential cellular basis been adequately studied. Nevertheless, the general pathological studies indicate that marked local reactions of the connective tissue occur. In fact, leaving out of account special lesions, such as the metaplasia and neoplasia of certain epithelial cells due to parasitic irritation and the hyperplasia of cells in contact with parasites as encountered in the bile ducts in clonorchiasis, the outstanding reaction to worm parasites predominantly involves the connective tissue. In addition some of the work strongly suggests an actual defense by these cells. For example, the recent work on the pathology and etiology of filariasis (*Wuchereria bancrofti*) by O'Connor and Hulse (1932) and O'Connor (1932) is very suggestive from the standpoint of the present discussion. These authors found that within lymphatic vessels containing living filaria a chronic oblitera-

tive lymphangitis develops and is associated with polypoid growths protruding into the lumen of the vessels above and below the worm which result from a proliferation of endothelial and other connective tissue cells. As obliterative changes take place the worm dies and degeneration proceeds in one of several ways. Calcification may ensue during which the worms are infiltrated with lymphocytes and plasma cells (mononuclear exudate cells) followed by a walling off with giant cells and large mononuclear wandering cells and the eventual invasion and walling off with fibroblasts. In other cases the picture may resemble a caseating tubercle. There is an intense infiltration of lymphocytes with some plasma cells and other mononuclear wandering cells. Foreign body giant cells appear and at the periphery of the reaction eosinophiles are numerous. Later fibroblasts appear and in time the whole area is fibrosed. This brief review tends to bring out the fact that the same cells that bear the brunt of the immune reactions in protozoan and other infections are the ones associated with the degeneration of the filaria. From the standpoint of the present discussion it would be of immense importance to know the rapidity and extent of these reactions in normal as compared to previously infected and hence possibly immunized persons.

As a rule the acquired helminthic immunities have not been associated with demonstrable protective antibodies. Extreme caution must, however, be exercised in excluding antibodies because most of the immunities are probably local (see, for example, Bachman and Molina, 1933) and may involve antibodies produced locally which when diluted in the blood stream are not demonstrable. In fact it is interesting that the one clear-cut case of a passive transfer of a helminth immunity is the work of Miller and Gardiner (1932) on the cysticercus of *Taenia taeniaeformis* where the parasite lives in the liver which is rich in macrophages and is strategically placed to pour antibodies into the blood. In this connection mention should also be made of the peculiar method of antibody action shown in the studies of Blacklock and Gordon (1927) and Blacklock, Gordon and Fine (1930) on acquired immunity of guinea pigs to *Cordylobia anthropophaga*. They showed in their first paper that a striking local immunity developed in the skin after its penetration by the fly larvae. This local immunity, which tended to spread from the original site, was found in new skin growing on an abraded immune area and persisted (in one experiment) in skin transplanted from an immune to a non-immune guinea pig. In their second paper, they showed that although no precipitins could be demonstrated to certain parts of the larvae, definite precipitins occurred for the haemocoele fluid and excreta of the third instar and that these by producing a precipitate in the gut of and around the larvae, blocked the gut, stopped the development of the larvae and thereby caused their death.

Before leaving the possible rôle of the connective tissue in helminth immunity, the remarkable infiltration of eosinophiles at the point of attachment of worms and around larvae should be considered. Parenthetically, it should be pointed out that eosinophilia is not an exclusive characteristic of infections with helminth parasites, but occurs in many cases where foreign proteins are absorbed and especially in diseases associated with protein sensitization. Asthma and various skin diseases due to bacteria are outstanding examples. The significance of eosinophiles around a worm or a portion of a worm is not clear, but evidence to date strongly suggests that they occur under conditions of sensitization or imnization of the host by protein antigen and are associated with the defense against foreign toxic protein. In fact, the work of Weinberg and Séguin (1915) suggests that they actually absorb or denature the foreign protein. (See also review in Taliaferro, 1929, and recent work of Stumberg, 1933.)

PARASITICIDAL ACTIONS OF CERTAIN NORMAL SERUMS, IMMUNE SERUMS AND DRUGS

Closely related to the defense reactions of the macrophages is the part they play in the action of certain protective and therapeutic serums, both normal and immune, and of drugs. Unfortunately, however, the exact method of macrophage action in these cases is not fully understood.

Following the original work of Laveran in 1902 a very extensive bibliography has accumulated showing that the serum from the majority of normal human beings is definitely protective and curative against many of the trypanosomes which produce diseases in domesticated animals when tested in mice. Of particular interest is a series of papers by Rosenthal and his collaborators (see review in Linton, 1929, and Taliaferro, 1929). They showed that normal human serum was rendered comparatively ineffective after repeated doses of it prior to the protective test, after splenectomy, after blockade with saccharated iron oxide and especially after a combination of splenectomy and blockade. These observations indicated that the reticulo-endothelial or macrophage system played a definite rôle in the activation of the human serum. Furthermore, that they played a unique rôle was indicated by the fact that the almost total destruction of circulating leucocytes by treatment of the mice with thorium-X did not lessen the protective property. Taken in conjunction with experiments showing the inactivity of such serum *in vitro*, Rosenthal and his co-workers concluded that such serum was not trypanocidal *per se* but was trypanocidogenic, *i.e.*, it had the power to stimulate the macrophage system to produce trypanocidal substances. In 1930 Yorke, Adams and Murgatroyd invalidated this latter contention provided the parasiticidal mechanism is similar *in vivo* and *in vitro* by showing that human serum is actually trypanocidal *in vitro*.

With the assistance of Miss Pavlinova (unpublished) I have been studying the protective action of normal sheep serum on *Trypanosoma duttoni* in the mouse, which was discovered by Thiroux in 1909, but as yet have obtained no clear-cut answer to these problems. For example, we have found as did Yorke, Adams and Murgatroyd for normal human serum, that normal sheep serum is trypanocidal *in vitro,* but we have also found, as did Rosenthal and his co-workers, that such serum loses its protective action in mice previously splenectomized and blockaded with India ink or foreign serum. These experiments, of course, do not disprove the possibility that normal serum is metabolized into a specific trypanocidal substance *in vivo* even though it, itself, is trypanolytic *in vitro,* but they make it more or less unnecessary. The possibility is made all the more unnecessary by the fact that an intact macrophage system seems necessary for the action of *immune* serum which is a specific substance already manufactured by other macrophages. Thus, in *Trypanosoma equiperdum,* the speaker and Kuhn (1932), developed a specific anti-trypanosome sheep serum which would protect mice from infection in doses of about 0.3 cc. per 20 gms. mouse, but was ineffective in doses as high as 1.7 cc. in mice which were just splenectomized and blockaded with India ink or foreign serum. This has also been shown for certain cases of passive immunity to bacteria. The most tenable hypothesis that has occurred to me to explain the action of the macrophages in normal and immune serum-therapy is that serum when introduced into normal mice is concentrated in organs rich in macrophages, such as the spleen and liver, and in these sites of high concentration kills the trypanosomes, whereas when introduced into splenectomized and blockaded mice it is diluted to such an extent as not to be effective.

The fact that an intact macrophage system is necessary for passive immunization indicates that not only do the macrophages secrete antibodies, but are necessary for the utilization of antibodies from another animal.

It is not the province of this paper to discuss the various theories of chemotherapeutic action, but it appears that many drugs, particularly those used for blood infections, are active only if the macrophage system is intact. The original work of Jungeblut (1927), Feldt and Schott (1927) and Kritschewsky and Meersohn (1926) (see review in Jungeblut, 1930) have been followed by many others showing that the macrophage system plays an essential part in the action of specific drugs within the body. Most of this work has been on mice infected with trypanosomes, spirochaetes and certain bacteria. It is consistent in showing that a given dose of drug which will cure a normal mouse of an infection is decreased in efficacy if the animal is splenectomized and blockaded.

Just what the macrophages do is still a puzzle and very probably may vary with the specific drug. The final explanation may include one or

more of the following: The drug may be changed to a more potent compound; there may be a cooperative immune response or the drug may be stored or concentrated by the macrophages. The last hypothesis is similar to the one suggested for the action of the macrophages in serum therapy.

CONCLUSIONS

In conclusion, I wish to stress the fact that evidence is rapidly accumulating to show the following: The cells of the blood and connective tissue, besides their manifold normal functions, are primarily concerned in all types of defense reactions of the host and are the basis for acquired and natural immunity except for such mechanical barriers as thickness of skin, body temperature and the like. They are probably active because of such characteristics as their widespread distribution, their phagocytic and digestive powers, and their developmental potencies. Of all these cells the polymorphonuclear leucocytes and macrophages are most directly involved and of these the macrophages apparently bear the brunt of the work while the smaller cells of the lymphoid-monocytoid series probably form a mesenchymal reservoir from which macrophages can be developed.

In acquired immunity, as distinguished from natural immunity, the macrophages take an even more prominent part and are generally more numerous, individually more reactive, react specifically, and in some, if not in all cases, produce local antibodies which, in turn, are correlated with the greater and more specific activity. The distinction between local and general immunity is largely unwarranted because the so-called general immunities result from the local reactions of the macrophages in strategically placed organs.

Furthermore, not only are the macrophages the most prominent in immune reactions, but seem necessary for the full utilization of certain normal trypanocidal serums, immune serums and drugs used in the chemotherapy of blood infections.

It is quite significant that the same cells are involved, in varying degrees, whether the animal be responding to a local or to a general invasion by protozoa, helminths, bacteria or other infectious agents, to immunizing procedures or to the introduction of sterile foreign substances. These facts although giving different pictures and involving different immediate mechanisms, pointing, as they do, to the unity of defense reactions in general, give little support to those who would postulate special systems for bacterial immunity as opposed to protoozal immunity, or bacterial and protozoal immunity as opposed to helminthic immunity.

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SEASONAL INCIDENCE AND CONCENTRATIONS OF SAND FLY LARVAE, *CULICOIDES DOVEI* HALL, IN SALT MARSHES (CERATOPOGONINAE: DIPTERA)*

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Sand flies are a source of intense annoyance to residents along the South Atlantic and Gulf Coast during certain seasons of the year. These tiny blood-sucking pests seriously interfere with the success of beach resorts and business establishments catering to tourists. The information and suggestions furnished in this paper should be helpful to drainage engineers, residents, and other persons interested in the development of water-front properties.

The investigations reported herein show that seasonal concentrations of sand fly larvae, *Culicoides dovei,* occur in wet soil shaded by trees, in ditches, near barriers, and in depressions of salt marshes. The seasonal incidence of larvae in these locations suggests that drainage ditches may either dispatch sand fly larvae to deep water or so concentrate them that during certain seasons they may be destroyed by natural factors or artificial treatments.

METHODS OF STUDY

The method used in isolating sand fly larvae from samples of marsh soil was reported in a preliminary paper (1). It furnishes a standard for measuring infestations of marsh soil and makes possible the comparing of infestations in different locations and at different seasons of the year. When a large number of samples were taken at a depth of 1 inch below the surface of the marsh soil, it was unusual to find in them more than an average of 2.4 larvae per quart of soil. When, however, samples were taken at the very surface of the soil of comparable locations, they furnished from 25 to 130 larvae to each quart of soil. The authors therefore standardize their samples by using 1 quart of wet surface soil from an area measuring 1 square foot on the surface of a marsh. This represents a depth of much less than 1 inch.

The soil sample is thoroughly mixed by pouring or by stirring and is allowed to settle overnight in a crystallizing dish. On the following morning the dish is placed in a larger dish. Tap water is carefully poured inside the edge of the larger dish until the smaller dish and soil sample are completely submerged. The tap water stimulates the larvae

* Contribution from the Division of Insects Affecting Man and Animals, Bureau of Entomology, U. S. Department of Agriculture. The writers are indebted to D. G. Hall for the illustrations and for his valuable assistance.

and after 3 days almost all of them have migrated into the moat formed between the two dishes. From this location the larvae are easily removed with a pipette. Experiments have shown that this method of isolation obtains 95.3 per cent of the entire number present in quart-size samples. The figures used in this paper are the numbers of larvae actually isolated from the samples, ignoring the few not recovered.

Wet soil from under the shade of trees of salt marshes or from ditches traversing the marshes is used in rearing sand fly larvae. It is heated in an oven to kill any larvae present and is then saturated with "clean" marsh water. Small quantities of it are placed in chemically clean Erlenmeyer flasks, and when the soil has settled to the bottom the surface water is removed with a pipette. After the samples are cool, sand fly larvae and free living worms are added with a pipette, and the flasks are placed in a tilted position in wet sand. This leaves the soil soft enough to permit the larvae to migrate in it, but firm enough to retain a slanting surface, so that the larvae can find the degree of moisture desired by them. They usually conceal themselves near the surface, leaving only the head exposed.

THE DEVELOPMENT OF SAND FLY LARVAE

The reactions of sand fly larvae to wet soil were strikingly illustrated in the following experiments: When a cloth bag containing marsh soil was suspended in water where larvae were kept, the larvae penetrated the meshes of the cloth and remained in the soil. When small quantities of soil or pieces of debris were placed in the water, the larvae attempted to conceal themselves among the particles.

Under experimental conditions the minimum period required for development of small sand fly larvae in wet soil was 4 months and 20 days. Under similar conditions, collections of larger larvae of the same species produced sand flies for periods of from 4 to 7 months. In one experiment all the larger larvae were reared to maturity, although in most of the tests only about one-half of the larvae produced adults. The reared sand flies were identified as *Culicoides dovei* Hall.

The factors which stimulate the larvae to pupate are considered important, but are not fully understood. Some salt-marsh species and one species developing in rot holes of trees pupate and emerge when free water is allowed to evaporate from their breeding places. In the laboratory some larvae pupate on the sides of the jars just above the edges of the water. In nature it is observed that great numbers of sand flies emerge just after the season of high tides passes. These observations suggest that when larval concentrations are disseminated by high tides, some larvae are left aground and become partially dry. They suggest that partial drying stimulates the larvae to pupate and that emergence of sand flies follows within a few days.

SEASONAL INCIDENCE OF LARVAE IN THE SHADE OF MARSH TREES

In the previous paper (*1*) it was shown that concentrations of sand fly larvae of different sizes were recovered from the wet soil of shady areas near the edges of grass marshes. (Fig. 1.) They were found in

Fig. 1. Concentrations of sand fly larvae occur in wet soil along the edges of salt marshes where they are shaded by trees.

soil having the following characteristics: (1) salinity approximately that the sea water, (2) cool temperature, (3) constant moisture, (4) decaying leaves, (5) dense shade, and (6) a pH of from 6.5 to 7.0. From 31 to 165 larvae each were recovered from standard quart-size samples of muck. The larvae came from locations which were reached by the high tides for a few days during each month.

In the present studies, during a period of 12 months, October, 1931, to September, 1932, 182 1-quart samples of soil were obtained from shady locations on the edges of grass marshes in the vicinity of Charleston, S. C. These locations were identical with those described above. They contained wet soil, which was reached by walking from the high land. These collections averaged 15.25 larvae to the quart of soil. Measured by the averages of the collections for each month, the greatest concentrations in the shade of trees occurred between July 1 and November 30. (Fig. 2.) In this diagram it is seen that larvae were scarce in May and abundant in October. Both months were preceded by seasons of sand fly activity. In May the tides reached a maximum of 6 feet, 3

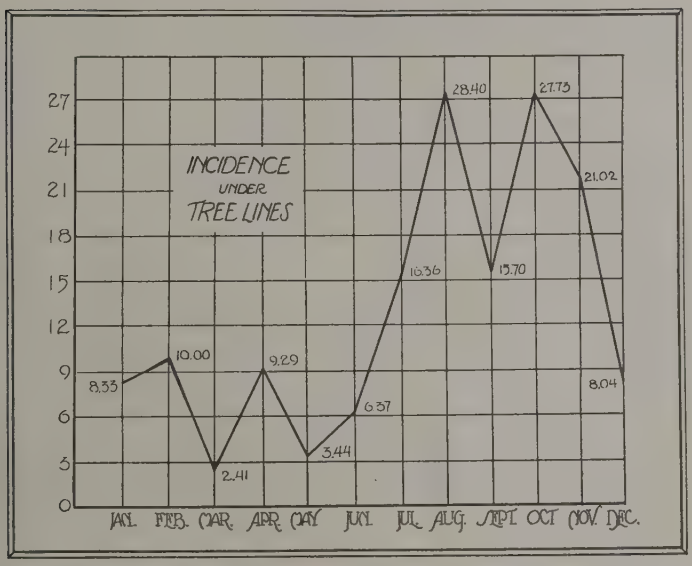

Fig. 2. In shady areas near the edges of grass marshes, which represent the high-water marks of the high tides of each month, the average number of sand fly larvae in quart samples of soil varies during different months of the year. The figures on the left represent the numbers of larvae per quart of soil.

inches. In October the highest tides were 6 feet, 2 inches. During May the rainfall was 1.12 inches. During October the rainfall was 0.71 inch. In May the mean of the lowest daily temperatures of the atmosphere was 66.1° F. and in October it was 62.3° F., yet for May the average of sand fly larvae was 3.44 to the quart of soil and for October 27.73 larvae. Evidently the tides, rainfall, and atmospheric temperatures occurring during the months of May and October do not account for the differences in the number of larvae obtained in the samples during these two months. The diagram (Fig. 2) shows, however, that the summer months, which normally have high atmospheric temperatures, precede a high incidence of larvae in the shade of trees; and that the winter months, which normally have low atmospheric temperatures, precede a reduction in the numbers of larvae in these locations. Since larvae are more abundant in the early part of the year in open grass marshes, the correlations suggest that during high atmospheric temperatures sand fly larvae are protected by the shade of trees along the edges of the marshes and that during low temperatures the open marshes are more suitable to their requirements.

With these fluctuations of larval abundance in mind, the writers were

able to predict, and later confirm, the occurrence of high concentrations of the larvae. On October 9, 1932, just after sand flies had shown much activity and following unusually high tides, samples of soil were obtained from the shade of trees near the edges of the marshes, and 47 1-quart samples, representing four different marshes, furnished an average of 133 larvae to the quart of soil.

DISSEMINATION OF LARVAE FROM THE SHADE OF TREES TO OPEN MARSHES

Since it is physically impossible to walk over soft open marshes and collect muck samples for sand fly larvae, studies were made along a ditch which crossed a row of shade trees and traversed a portion of a soft, unshaded marsh. During the high, or "spring," tides, water came into the ditch and was trapped there. On account of the abrupt termination of the ditch in the open marsh, the trapped water kept the soil wet between visits of the tides. From December 1, 1931, to March 31, 1932, 55 quarts of soil obtained from the unshaded part of the ditch were found to have an average of 97.29 larvae per quart, the monthly averages being, for December, 86.42; for January, 110.20; for February, 78.75; and for March, 174.50. If we compare these figures with those shown in Figure 2 it is seen that large concentrations of larvae were obtained in the unshaded part of the ditch shortly after there was a decrease in the incidence of larvae in the shade of trees. The differences in the incidences of the two locations clearly show tendencies of drainage to transport sand fly larvae from the shade of trees to the ditches of salt marshes.

Concentrations of Larvae along Roads and Barriers in Marshes

Roads and barriers across open marshes produce conditions favorable for large concentrations of sand fly larvae. Tidal water goes out slowly through culverts beneath road beds, and the edges of the water recede gradually. This enables sand fly larvae to anchor themselves in the wet soil. From holes of fiddler crabs about the edges of such locations an average of more than 20 larvae to the quart of soil has been obtained.

Some roads across marsh areas (Fig. 3) are not provided with culverts. The base of their construction consists of logs, limbs of trees, and rubbish. When such roads are inundated by the tides, and the lower ground back of them is flooded, there is no outlet for the water, and as a result the soil remains wet. Samples of soil obtained from such areas during the month of February furnished an average of 390 larvae to the quart.

The conditions furnished by roads through marshes are similar to those resulting from partly closed flood gates or those found in basins having open gates for "in and out" passages of tidal water.

Fig. 3. A road or dam-like construction hinders drainage of surface water in salt marshes and favors concentrations of sand fly larvae. In February the area shown in the photo had an average of 390 sand fly larvae to a quart of wet soil.

Concentrations of Larvae in Depressions and "Blind" Ditches

In depressions and ditches (Fig. 4) which are not provided with outlets for drainage there is a natural tendency for sand fly larvae to concentrate along the edges of the water and in adjacent holes of fiddler crabs. Both the depressions and ditches hold water after the tides recede, and as the water seeps into the soil or evaporates, small quantities of wet soil examined will show concentrations of sand fly larvae. At Charleston, S. C., the soil of "blind" drainage ditches contained from 200 to 400 larvae per quart, the greater numbers occurring when the water-level was very low. At Savannah, Ga., ditches ending in the open marsh furnished an average of 216 larvae in 1-quart samples of soil. Near Fort Pierce, Fla., the ditches of mangrove marshes furnished as many as 1,500 larvae in a single quart of muck. These observations made in different localities show that there is a natural tendency of drainage waters to concentrate sand fly larvae in low places of the marshes.

TIDAL CONCENTRATION OF SAND FLY LARVAE IN SEEPAGE MARSHES

In seepage marshes sand fly larvae are carried along with tidal water beneath the surface of the soil.

Seepage marshes may be recognized by the compact appearance of their surfaces, by the presence of short, salt water vegetation, and by the

Fig. 4. Some drainage ditches ("blind ditches") do not enter streams, but terminate on the open marshes. About their mouths, concentrations of sand fly larvae are found.

rise and fall of the daily tide in holes made by fiddler crabs. Beneath the solid-appearing mat of grass or pickle-weed roots there is encountered a soft subsoil. The crust seems sufficiently firm to prevent the passage of emerging sand flies, but since the marsh crust is tunneled by fiddler crabs, it is readily suggested that sand flies may emerge and escape through their holes. If the water in one hole is agitated with a Banta pipette, the soil in several of the holes may be stirred up, showing that they are connected.

Since only from 3 to 8 larvae per quart were commonly found in samples of soil obtained with a pipette from the holes of fiddler crabs, the importance of seepage areas as places for the development of sand fly larvae might not be appreciated. In order to obtain data on these areas, tightly constructed cages were used for the recovery of sand flies emerging from seepage marshes in the vicinity of Charleston, S. C. In the cages, which were placed over crab holes, specimens of *Culicoides melleus* and *Culicoides dovei* showed rather definite periods of emergence, with an overlapping of the periods, however, during which both species emerged into the cages at the same time. The occurrence in the cages seemed to agree with the prevalence of the two species in nature. The numbers of adults obtained from cages in the seepage marshes show that emergence occurring over extensive areas can account for hordes of adult sand flies.

The numbers of sand fly larvae developing in seepage areas were more fully understand when their incidence was studied in a "blind" ditch of such a marsh. During the daily tides water would seep in and then out again through the soil of the ditch. During the time of low tides only the lower end of the ditch contained water and wet soil, and at one such time this location furnished an average of 53 larvae to the quart of soil. The infestation was sprayed with creosoted pine sap, and after a few days samples of the soil showed a reduction of 72 per cent in the larval population. Later samples showed an increase in the number of larvae until, two weeks after treatment, the population was approximately the same as before the soil was treated. This building up of the infestation in such a short time after treatment was not due to unusually high tides or rainfall. It could be interpreted only as the induction of new larval concentrations in the "blind" ditch by the seepage water of the tides. The experiment demonstrated the effectiveness of a ditch in concentration larvae from seepage water in marsh soil.

SAND FLY LARVAE IN MANGROVE MARSHES

Salt marshes differ in the extent of open marsh between the shade and the streams of water. In the grass type of marsh there are likely to be extensive areas between the trees and the streams. In the mangrove marshes, however, the trees extend to the edges of the streams and send entangled root systems into the inundated soil. In grass marshes it has been shown that the unusually high tides serve as a vehicle for transporting sand fly larvae from the shade to open marshes. In mangrove marshes the rise and fall of the daily tides amounts to only a few inches but even this appears sufficient to transport the larvae to deep water.

In numerous samples obtained near the edges of rivers bordered by mangrove marshes it was seldom that a sand fly larva could be obtained. Samples of soil collected from banks of ditches as close as 25 feet from the river contained only 3 small larvae to the quart. From sections of ditches containing water from the river during the entire year, 63 samples furnished an average of 2.6 larvae per quart of soil. Nine samples from sections of these ditches above the normal daily tide marks, however, averaged 89.1 larvae per quart of soil. Where the ditches were partly filled with muck and where there was no tidal action whatever (Fig. 5) the soil samples furnished as many as 271 larvae to a quart of soil.

Seasonal Incidence in Ditches

Since decided concentrations of larvae were encountered in ditch soil above the normal tide lines in mangrove areas, it was interesting to study the incidence during different months and at different distances from the ditches, in the marshes near Fort Pierce, Fla. For a period of one year

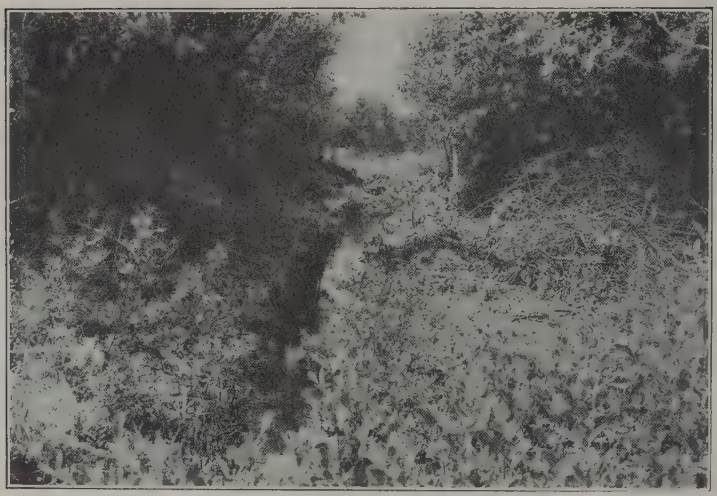

Fig. 5. In drainage ditches of mangrove marshes, concentrations of sand fly larvae occur in wet soil above the marks normally reached by high tides.

soil samples were collected from favorable locations in drainage ditches of a mangrove marsh. When the larvae were isolated and averages were made according to the months of collection, the figures clearly indicated that the greatest concentrations of larvae occurred during January, February, and especially March, when the larvae averaged 99 to the quart of soil. (Fig. 6.) During the month of April the number of larvae decreased to 37 per quart, and the low point was reached in June, when only 47 larvae were isolated from 48 quarts of soil. These figures show that the abundant occurrence of larvae in drainage ditches of mangrove marshes compares with that of ditches in unshaded grass marshes. In both types the marsh areas drained by ditches were inundated by the unusually high tides of each month.

Relative Populations at Different Distances From the Ditches

From January to June, inclusive, soil samples were taken at different distances from the water in drainage ditches of mangrove marshes. The numbers of larvae found at each distance were averaged, and it was found that within the ditch, under the water and from the banks not reached by the water, there was an average of 87.3 larvae to the quart of soil. Measuring from the edge of the water, in the first 5 feet there was an average of 190.6; from 5 to 10 feet, an average of 44.83; from 10 to 50 feet, an average of 6.36; from 50 to 100 feet, an average of

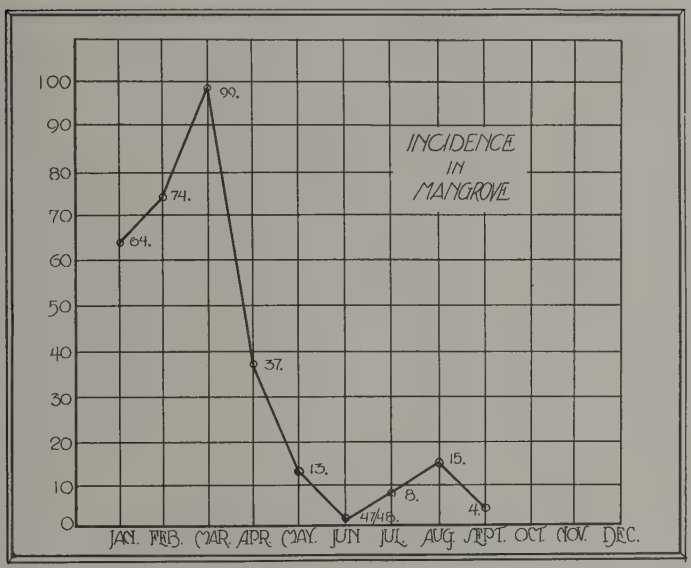

Fig. 6. In ditches of mangrove marshes, which are inundated during the unusually high tides, the average number of sand fly larvae in quart samples of soil varies during different months of the year. The figures on the left represent the numbers of larvae per quart of soil.

1.99; and from 100 to 300 feet from the ditch there was an average of 1.36 larvae to the quart of soil. Of all the larvae found within 300 feet of the ditch, approximately 95 per cent were within 10 feet of the edge of the water. (Fig. 7.)

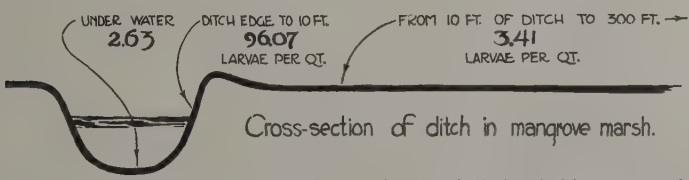

Fig. 7. In mangrove marshes, the wet banks of ditches hold concentrations of sand fly larvae. The diagram illustrates the location and average number of larvae in quart samples of soil at different distances from the ditch for the period of January to June, inclusive.

THE SIGNIFICANCE OF THESE STUDIES

The seasonal concentrations of sand fly larvae in ditches of different types of marshes suggest that tidal action in good ditches would undoubtedly dispatch many of the larvae to deep water. Ditches through

seepage marshes apparently offer a means of concentrating the larvae in such marshes into places where they can be treated. During periods of seasonal concentrations of larvae in ditches near resorts, it seems desirable to unite the two methods and increase the efficiency of drainage ditches by artificial treatments for the infested soil. The treatments should be directed to portions of the ditches which contain wet soil.

It is not easy to construct satisfactory drainage ditches through soft marsh areas so that their terminals will continue to drain the marshes into streams of running water. The ditches which engineers are constructing at different places through the soft marshes for the elimination of breeding places of salt marsh mosquitoes should concentrate and reduce the breeding of sand flies. Low places in the ditches are especially favorable for sand fly larvae, and whether or not the desired degree of control can be obtained only by drainage is a matter for further study rather than one for speculation.

SUMMARY

1. Sand fly larvae from the soft soil and shaded areas and from ditches, barriers, and depressions of both grass and mangrove marshes were reared and identified as *Culicoides dovei*.

2. From July 1 to November 30, concentrations of sand fly larvae occur in the shade of trees near the edges of grass marshes at Charleston, S. C.

3. As the incidence of sand fly larvae decreases in the shade of marsh trees, it increases in ditches of grass marshes leading from such areas.

4. On the wet banks of roads and barriers, in depressions and ditches, and in "blind" ditches of seepage areas of salt marshes, there are natural tendencies toward concentrations of sand fly larvae.

5. In ditches of mangrove marshes, the seasonal incidence of concentions of sand fly larvae is comparable to that of ditches in grass marshes. Both types of marshes are completely inundated by the unusually high tides.

6. In mangrove marshes, isolations of sand fly larvae from soil samples taken within 300 feet of ditches show that approximately 95 per cent of the larvae are within 10 feet of the edge of the water.

7. The seasonal concentrations of sand fly larvae along ditch banks suggest locations and periods when artificial treatments could be used to supplement the control afforded by ditch drainage.

8. Whether or not tidal action without treatments of drainage ditches can furnish the desired degree of control of sand flies is a matter for further study.

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LIFE HISTORY STUDIES ON *COTYLOPHORON COTYLOPHORUM* (FISCHOEDER, 1901) STILES AND GOLDBERGER, 1910

By Wendell H. Krull

Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture

Le Roux (1930), in a preliminary report on the life cycle of *Cotylophoron cotylophorum* (Fischoeder, 1901) Stiles and Goldberger, 1910, stated that in places where this parasite was found in abundance in cattle and sheep, snails of a species probably identical with *Bulinus schakoi* (Syn. *Isidora schakoi*) were plentiful. While the foregoing statement suggests that *Bulinus schakoi* is an intermediate host of this amphistome in South Africa, it affords no actual evidence as to the life history.

In a recent paper by Krull (1932), *Cotylophoron cotylophorum* was reported from Puerto Rico, and it was shown by experiments that in the United States a snail, *Fossaria modicella* Say,[1] was capable of serving as an intermediate host. It is the purpose of the present paper to outline the life history of *C. cotylophorum* as determined by experiments.

MATERIAL

The eggs of *C. cotylophorum* used in the following experiments, as well as the adult flukes on which the species determination was based, were collected by Dr. M. C. Hall, Chief, Zoological Division, Bureau of Animal Industry, at Mayaguez, Puerto Rico, April 11, 1932. The eggs on arrival were put in small covered stender dishes in fresh water; some were kept in the laboratory and others in the refrigerator. Some of the eggs kept in the laboratory were hatching on May 10, 1932; hatching subsequent to this date was regulated by adjusting the temperature at which the eggs were kept.

The snails used for the experiments were from two lots, one taken by Dr. L. E. Swanson in Utah, May 14, 1932, and the other laboratory-raised from parent stock collected in Utah on March 28, 1932. The snails used for experiment and the snails used as controls were kept in covered moist chambers of two-liter capacity filled with water to a depth of 3 inches, and supplied with partially decayed lettuce for snail food. Since all the stream, pond and spring water is acid in the vicinity of Beltsville, Md., it was necessary to add calcium carbonate to the aquaria, both the technical grade and the precipitated chalk being suitable for this

[1] Identified by Dr. F. C. Baker, University of Illinois, who stated that these are *Fossaria* sp., "somewhat like *modicella* Say." Since these snails are abundant in parts of Utah and have been sent to us from that State in large numbers, there seems to be little doubt as to the identity of the snails.

purpose; a sufficient amount was added to cover the bottom of the aquarium.

From a large consignment of snails collected May 14, 1932, in Utah, approximately 100 snails were crushed and examined for trematode infections. All of these snails were negative and may be considered as a control in addition to the control described subsequently.

EXPERIMENT WITH MATURE SNAILS TAKEN IN NATURE

In this experiment 21 mature snails, collected May 14, 1932, in Utah, were isolated in an aquarium, as already described, and numerous miracidia of *C. cotylophorum* were added. As a control in this experiment, 40 snails were used and of these 24 lived for more than a month; all were negative for all trematode infections. No attention was given to the snails in the experiment, except to feed them lettuce, until June 15, 1932, when it was noticed that they were crawling out of the water; the aquarium was cleaned and 12 of the original 21 snails were still living. One was examined by dissection; its liver was filled with rediae and developing cercariae, of which some were large enough to exhibit motility. These cercariae had a heavy network of pigment in the region of the eyespots. The remaining snails were cleaned with a brush and examined under a compound microscope; and cercariae were seen in 4 of the specimens. The first cercariae were shed by 5 snails June 19, 1932; 2 more snails began shedding a day later; and all were shedding June 22, 1932. Thus a total of 12 snails were infected, of which 11 lived to shed cercariae for about 6 weeks. A detailed account of the shedding of cercariae is given in the following table.

The table shows that all the snails began to shed at approximately the same time, but that the shedding period, which was terminated by death, was variable. It also shows that about half of the cercariae escaped during the first 2 weeks. A number of factors are, no doubt, responsible for the great variation in the number of cercariae escaping from day to day as is shown, for example, by the number shed on July 1 and 2. Temperature appears to be an important environmental factor in influencing the escape of cercariae, since it was noted that in warm weather the number increased, while in both cool and extremely hot weather, if prevailing for more than a day, the number escaping was retarded.

In connection with the total number of cercariae which developed in the snails, it is interesting to note that Takahashi (1928) stated that in *Paramphistomum cervi* the sporocyst produced 9 rediae, each of which gave rise to 20 cercariae. Thus, according to this author, each snail, theoretically, produced 180 cercariae. In *Cotylophoron cotylophorum* the average number of cercariae produced was 152 per snail, which corresponded fairly closely to the number reported by Takahashi for *P. cervi*.

TABLE 1.—*Number of Cercariae Shed by the Snails*

Date	Number of Snails Shedding	Number of Cercariae Shed
June 19	5	20
20	7	41
21	9	63
22	11	97
23	11	138
24	11	190
25	11	82
26	11	22
27	11	23
28	11	97
29	11	72
30	11	10
July 1	11	10
2	11	271
3	11	73
4	10	69
5	10	7
6	10	38
7	10	0
8	10	25
9	10	0
10	10	2
11	10	7
12	10	1
13	8	9
14	8	2
15	8	56
16	7	52
17	7	43
18	7	54
19	7	52
20	7	9
21	5	0
22	4	14
23	2	3
24	2	0
25	2	0
26	2	7
27	2	0
28	2	0
29	2	5
30	2	2
31	2	1
August 1	2	4
2	1	0
3	1	2
4	1	0

Total number of cercariae produced, 1673
Average number produced per snail, 152

The time at which cercariae were shed daily and the daily period during which they were shed were found to be quite regular. Shedding was found to take place from 7: 30 a. m. to 1 p. m., the peak usually being about 8: 30 a. m. Cercariae were found to escape only occasionally at other hours of the day; if large numbers escaped from a snail at odd hours, it indicated that the snail was about to die. The period during which the cercariae escaped increased with the increase in the number of cercariae, as was reported by Krull and Price (1932) for *Diplodiscus temperatus* Stafford.

After the cercariae escaped from the snail they swam about in the water quite actively and encysted within an hour, encystment occurring on vegetation or the walls of the container. The cysts are gray when examined on a white background and appear to have a pearly luster when

examined on a black background. The cyst is round on surface view, and its spherical diameter is somewhat less than the diameter at the attached base, since it flares somewhat at the base. In fresh material the average measurements are: cyst diameter, 180 μ; base diameter, 210 μ; cyst height, 125 μ.

The cercariae which encysted on the lettuce or on the sides of the glass container were unevenly distributed; those on the lettuce were usually crowded in the folds of the leaf, while those on the container were near the top and on the side where the light was more intense. The firmly attached cysts were easily removed with a teasing needle or with the tip of a pipette.

LIFE SPAN OF ENCYSTED METACERCARIAE

The following observation indicates that encysted metacercariae live for a considerable length of time. On July 2, 1932, 16 cercariae were transferred to a covered stender dish in which the cercariae encysted on the bottom because of the small amount of water in the container. The container with the cysts was set aside and examined at least once a week. Movement of the metacercariae within the cyst was the criterion for determining whether the metacercariae were alive; the dead parasites were easily distinguished by other characteristics such as the color of the encysted parasite and the organization and distribution of pigment and excretory granules. The encysted metacercariae began to die November 15, 1932, and the last one was dead December 2, 1932. Though the metacercariae were relatively quiescent during these weeks of encystment, the approach of death was marked by a very decided increase in activity of the metacercaria within the cyst. This experiment indicates that the average life of the encysted metacercariae is about 5 months under what, so far as we know, may be called ideal conditions.

EXPERIMENT WITH YOUNG LABORATORY-RAISED SNAILS

In addition to the infection experiments with snails taken in nature, experiments were carried out with laboratory-raised snails. A number of snails from 1 to 10 days old were isolated individually in small watch glasses, (U. S. Bureau of Plant Industry Model) and 3 miracidia were added to each container. The miracidia promptly attached themselves either to the regions around the head or to the mantle of the snail, and some penetrated within 15 minutes. After the snails had been exposed to attack by miracidia for 2 hours they were transferred to an aquarium and cared for as previously described. All snails became infected, but none lived long enough to shed cercariae. Fifteen days after exposure to the attack of miracidia, one of the snails was examined by dissection and a small sporocyst containing developing rediae was recovered from the liver; one redia had developed sufficiently to show the pharynx and

rudimentary intestine. The remaining snails were examined through the shell with a compound microscope and the spire cavity, normally occupied by liver, was found to be filled with developing rediae, only the proximal part of the liver remaining intact. All the snails, except one described later, either died at this stage of infection or lost their infections and died within 3 days. The exceptional snail was given the very best possible care and was examined daily from June 17, 1932, until it died on July 1, 1932. This snail contained a rather large number of rediae which completely filled the spire. During the time that the snail was under observation the rediae did not grow, but they had destroyed all of the liver by the time the snail died. No fibrous bands or adhesions were observed in the spire cavity such as were recorded by Barlow (1925) in his interesting observations on *Fasciolopsis buski* (Lankester).

The snails made little or no growth after having been infected and, although this experiment was not of sufficient magnitude to be conclusive, it seems reasonable to assume that the amount of food necessary to mature an infection is either not sufficient or does not increase fast enough in a developing snail when infection takes place while the snail is very young.

INFECTION OF FINAL HOST

Many of the encysted metacercariae obtained from the experimental infection of fully grown snails were fed to 2 calves which had been raised at Beltsville, Maryland. *C. cotylophorum* does not occur in this vicinity and has not been previously reported from the United States, but to make certain that no contamination was present, 10 fecal samples obtained from each calf were examined for fluke eggs; all examinations were negative.

Calf No. 1, a Holstein heifer 3 months old, was given a total of 615 encysted metacercariae over a period of 3 weeks beginning June 19, 1932. Eggs appeared in the feces of the calf in the latter part of the fourth month of infection and on October 13, 1932, eggs were quite numerous.

The feces were washed through graded screens and sedimented until 5 to 10 cc. of sediment was left for examination. The sediment in water was distributed in 5 watch glasses and examined with a binocular dissecting microscope, using a combination of 8x oculars with 4x objectives; the eggs are easily seen with this magnification and the field is relatively large.

Calf No. 2, a roan bull calf a month older than calf No. 1, was given a total of 238 encysted metacercariae during the week beginning June 23, 1932, and, on October 15, 1932, eggs appeared in the feces in small numbers. The calf was killed and examined postmortem on January 12, 1933, and 6 adult specimens of *Cotylophoron cotylophorum* were recovered from the rumen.

EXPERIMENT TO DETERMINE TIME NECESSARY FOR HATCHING OF EGGS

Since calf No. 1 had an infestation with *Fasciola hepatica* in addition to that with *C. cotylophorum* it was not only possible to ascertain the time necessary for the miracidia of *C. cotylophorum* to mature and hatch, but, also to obtain a comparison of the eggs of the two species of flukes. Fifteen eggs of *F. hepatica* and 11 eggs of *C. cotylophorum* were isolated with a pipette from a fecal sample taken October 13, 1932, put in a covered stender dish in clean water, and kept at laboratory temperature. Miracidia could be recognized in the *F. hepatica* eggs on October 26, 1932, and in the *C. cotylophorum* eggs a day later. The eggs of *F. hepatica* hatched from October 31, 1932, to November 4, 1932, inclusive, and those of *C. cotylophorum* hatched November 10, and 11, 1932.

This experiment shows that *F. hepatica* eggs hatch sooner than those of *C. cotylophorum* when kept under identical conditions, the former requiring about 3 weeks and the latter 4 weeks. Thus, on the basis of experimental evidence, approximately 6 months is required to complete the life cycle of *C. cotylophorum.*

EGGS OF *C. cotylophorum*

Fischoeder (1903), in his description of *C. cotylophorum,* gave the egg sizes as 125 to 135 μ long by 65 to 68 μ wide. Stiles and Goldberger (1910), Maplestone (1923), and Stunkard (1929) redescribed the worm, but did not give the egg sizes. The present writer finds the average measurements of 12 eggs teased from a preserved specimen of *C. cotylophorum* collected in Puerto Rico to be 126 μ by 61 μ, and the average measurements of 12 eggs collected from the feces of Calf No. 1 to be 132 μ by 68 μ. The average measurements of *Fasciola hepatica* eggs from Calf No. 1 were 143 μ by 81 μ.

Fig. 1. Photomicrograph showing the differences between the eggs of (a) *Fasciola hepatica* and (b) *Cotylophoron cotylophorum.*

The egg of the widely distributed *Paramphistonum cervi* might easily be mistaken for the egg of *C. cotylophorum* were it not for its size; Stunkard (1929) gives the egg measurements of *P. cervi* as 145 to 160 μ by 75 to 99 μ, Nöller (1929), in differentiating the eggs of *P. cervi* and *F. hepatica,* shows that the former are larger; the eggs of *C. cotylophorum* (Fig. 1, b), however, are smaller than those of *F. hepatica* (Fig. 1, a). In addition, the egg shells of *C. cotylophorum* are colorless and are somewhat attenuated at the opercular end, while those of *F. hepatica* are yellow and not attenuated.

SUMMARY AND CONCLUSIONS

Fossaria modicella Say has been found capable of functioning as a satisfactory host of *Cotylophoron cotylophorum,* and as this snail is widely distributed over the Western United States, it is evident that the parasite could become well established in that part of the United States should the parasite be imported into that area.

Infections were obtained in laboratory-raised snails as well as in snails taken in nature; in the former all the snail hosts died before cercariae escaped from them; in the latter cercariae were shed 32 days after infection. It is established that the latter infections were obtained experimentally and were not originally present, on the following grounds; this fluke is not known to be established anywhere in the continental United States; no infections developed in the control snails; all snails began to shed cercariae within three days of one another following simultaneous exposure to miracidia; and the findings in experimentally infected adult snails collected in nature coincided with the findings in artificially infected laboratory-raised snails.

The metacercariae encysted on vegetation and the sides of containers, and under favorable conditions lived for 5 months.

The flukes matured in calves infected with metacercariae, and eggs were recovered from the feces of calves 4 months after infecting them. Six adult specimens of *Cotylophoron cotylophorum* were recovered from the rumen of a calf which had been experimentally infected.

The eggs of *Cotylophoron cotylophorum* hatch in 4 weeks when kept at room temperature, or about a week later than eggs of *Fasciola hepatica* kept under the same conditions.

The eggs of *C. cotylophorum* may be distinguished from those of *F. hepatica* by color, shape and size; the former are colorless, ovate and smaller, the latter yellowish or brownish, oval and larger. *C. cotylophorum* eggs may be distinguished by their smaller size from those of *Paramphistomum cervi.*

As determined experimentally, the complete life cycle of *Cotylophoron cotylophorum* requires approximately 6 months, 1 month being re-

quired for the eggs to hatch, 1 month for the intramolluscan development to reach the cercaria stage, and 4 months for flukes to mature in the final host.

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ON SOME TAPEWORMS FROM THE BULLSNAKE (*PITYOPIS SAVI*), WITH REMARKS ON THE SPECIES OF THE GENUS *OOCHORISTICA* (CESTODA)

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For the material which forms the basis of the following paper I am indebted to Mr. H. R. Osheroff, of Omaha, to whom I wish to express my thanks. It consisted of a large number of complete strobilae, together with numerous fragments, all in an exceedingly good state of preservation. It was thus possible to ascertain with a fair degree of accuracy the limits of variation within a species. Co-type slides deposited with the British Museum; Molteno Institute, Cambridge; University of Reading; Bureau of Animal Industry, Washington; École de Parasitologie, Paris; Indian Museum, Calcutta.

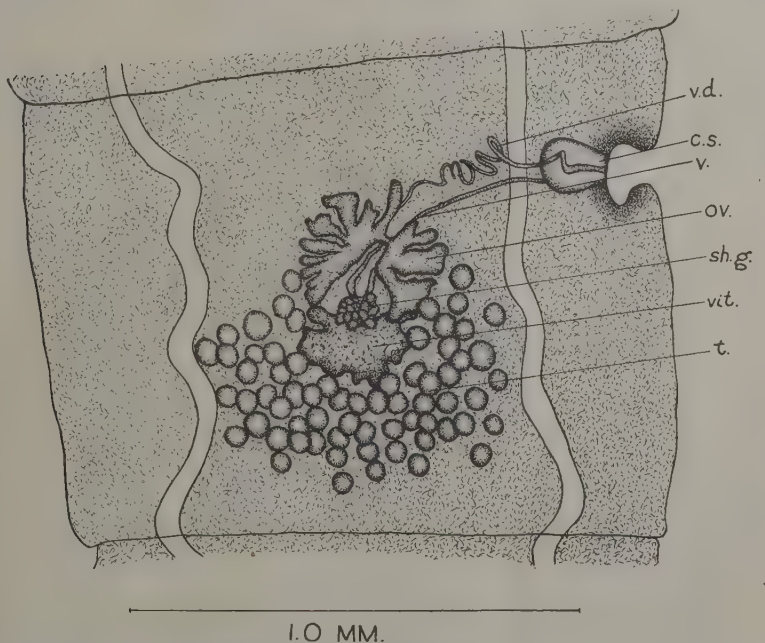

Fig. 1. Mature proglottis of *Oochoristica osheroffi* n. sp. Abbreviations, *c.s.*, cirrus-sac; *ov.*, ovary; *sh.g.*, shell-gland; *t.*, testes; *v.*, vagina; *v.d.*, vas deferens; *vit.*, vitelline gland.

Oochoristica osheroffi n. sp. (Fig. 1)

Length* 60–205, maximum breadth 1–1.4. Scolex 0.37–0.4. Mature proglottides very compressed. Genital organs not occupying the whole proglottis. Genital pore at limit of anterior quarter or fifth of proglottis margin, opening into a conspicuous genital cloaca. Cirrus-sac 0.132–0.24 × 0.07–0.118, in mature proglottides extending to excretory vessels, in gravid ones half crossing them. Testes 59–76, 0.048–0.08 × 0.036–0.072, extending anteriorly to middle of ovary. Onchospheres 0.024–0.042 × 0.022–0.036, cavities containing them 0.034–0.06 × 0.032–0.056. The table given later shows the differences between this form and others of the same genus.

O. fibrata Meggitt, 1927

A few isolated proglottides and one complete strobilus were found. They differed from the description already given in the following respects. Length 55–69, width 0.6–0.8. Scolex 0.46 dia. Proglottides more elongated. Cirrus-sac 0.156–0.24 × 0.082–0.114, often extending to centre of proglottis. Testes 41–64, 0.044–0.072 × 0.04–0.06, extending anteriorly to middle of ovary. Onchospheres 0.026–0.032 × 0.02–0.024, the cavities containing them 0.044–0.054 × 0.042–0.054.

Including the two forms just described, altogether 40 species of the genus *Oochoristica* have been recorded, to which number must be added *Catenotaenia symmetrica* Baylis, 1927, and *Atriotaenia parva* Sandground, 1926. The genus *Atriotaenia* Sandground, 1926, can in no way be distinguished from *Oochoristica* and should fall into synonomy. The characters employed in the descriptions have differed considerably according to the investigator. The course of the genital ducts is unalterable and therefore valid. Investigation of the material described above showed that the number of testes varied considerably, as did also the size of the cirrus-sac: both these characters therefore can only be used with discretion. The extent of the cirrus-sac across the proglottis and the arrangement of the testes afford a far safer means of discrimination, as it is difficult to see how any variations in the state of contraction of a proglottis can alter the relative positions of the genital organs. Baylis (1919, pl. xxi, figs. 6–7) gives figures of proglottides in two different states of contraction. In both cases the testes do not extend beyond the posterior margin of the ovary: as the excretory vessels are not shown, it is impossible to ascertain the extent of the cirrus-sac, though it should be noted that any force removing the longitudinal excretory vessel further from the margin of the proglottis would also stretch proportionately the cirrus-sac. Variations in length and width of strobilus cannot be considered as of much importance if unaccompanied by internal anatomical differences: *O. theileri major* and *minor* form a case in point. Unless fresh material be to hand, measurements of eggs and onchospheres should be regarded with suspicion. Onchospheres are capable of as much contraction or expansion as the strobilus. Inside the proglottis it is difficult to distinguish the outer shell of the egg from the cavity containing it and a confusion of the two is thus always possible. Of the

* All measurements in mm.

42 species Baer (1927, 161) considers *O. brasiliensis* (Janicki, 1904), *O. jheringi* (Zschokke, 1904) and *O. marmosae* Beddard, 1914, as synonyms of *Linstewia jheringi, O. dasjuri* (Beddard, 1914) as belonging to the genus *Anoplotaenia, O. rotundata* (Molin, 1859), *O. pseudopodis* (Krabbe, 1879), and *O. truncata* (Krabbe, 1879) as synonyms of *O. tuberculata* (Rudolphi, 1819), *O. sigmoides* Moghe, 1926, as a synonym of *O. crassiceps* Baylis, 1919, *O. murina* Zschokke, 1904, as a synonym of *O. didelphydis* (Rudolphi, 1819), *O. cryptobothrium* (Linstow, 1904) as a synonym of *O. rostellata* Zschokke, 1905, and *O. wagneri* Janicki, 1904, as a synonym of *O. tetragonocephala* (Bremser, 1856), recording in all 22 species. With the exception of *O. cryptobothrium,* the above synonomy may be accepted. According to Fuhrmann (1927, 396) the cirrus-sac of that species is 0.08 long, and the testes "liegen ganz dorsal in einfacher Lage, links und rechts von den weiblichen Geschlechtsdrüsen, und wenig hinter denselben", while according to Baer (1927, 176) the cirrus-sac of *O. rostellata* is 0.27–0.3 long and the testes "situés en arrière et de côté des glandes sexuelles femelles." Combined with minor discrepancies, these differences appear to exceed the limits of specific variation and the two species should be regarded as distinct. Of the species so accepted and others described later, *O. bivittata* Janicki 1904 is distinguished from all others by the small number of the testes, and *O. megastoma* (Diesing 1850) and *O. amphisbaenae* (Rudolphi 1819) by well marked characters in every case. *O. didelphydis* (Rudolphi 1819), *O. herpestis* Kofend 1917, *O. ichneumontis* Baer 1924, *O. megastoma* (Diesing 1850), and *O. surinamensis* (Cohn 1902) have the genital ducts passing dorsally to the excretory vessels instead of between them, and may be separated from one another in the case of *O. surinamensis* by the large size of the cirrus-sac, in that of *O. didelphydis* by the anterior position of the testes, and the remaining two by the number and position of the testes. *O. trachysuri* (MacCallum 1921) and *O. theileri* Fuhrmann 1924 are the only two species with the testes arranged in two isolated groups: considering forms *major* and *minor* of the latter the same species as stated by Fuhrmann (1924, 509), there exists no difference between the two species except the number of testes, while in all respects there is a close correspondence. In view of the general inaccuracy of the descriptions of MacCallum, no reliance should be placed on a single discrepancy and *O. theileri* falls into synonomy with *O. trachysuri,* the diagnosis of the species being that given by Fuhrmann. The presence of testes in two groups and of a cloaca lined by cuticular spines sufficiently separates this form from all others. *O. brasiliensis* Fuhrmann 1927 differs from *O. agamae* Baylis 1919, both from Lacertilia, only in the size of the eggs and the lesser development of the genital cloaca and should therefore become a synonym: *O. breslaui* Fuhrmann

TABLE I. *A comparative table of the species of Oochoristica*

Species	Host	Length	Breadth	Scolex diameter (in μ)	Sucker diameter (in μ)	Extent of cirrus-sac
agamae Baylis 1919	Lacertilia Ophidia	80	1.5	450–650	175	? to excretory vessel
ameivae (Beddard 1914)	Lacertilia	25	1.5			to excretory vessel
amphisbaenae (Rudolphi 1819)	Lacertilia	20–30	1.2	850–900	230	nearly to oporal margin
amphisbeteta Meggitt 1924	Carnivora	15–40	0.45	210		well past excretory vessel
biwittata Janicki 1904	Marsupialia	55	0.9	400		not reaching excretory vessel
brasiliensis Fuhrmann 1927	Lacertilia	120	1.0			just past excretory vessels
breslaui Fuhrmann 1927	Lacertilia	40	0.8	140	54	? to excretory vessel
crassiceps Baylis 1920	Lacertilia Ophidia	28–80	0.8–1.4	200–1,000	30	
cryptobothria (Linstow 1904)	Ophidia	130	2.5	600	250	
didelphydis (Rudolphi 1819)	Marsupialia	15	0.7			well past excretory vessels
erinacei Meggitt 1920	Insectivora Rodentia	15	1.0	330	165	well past excretory vessels
fibrata Meggitt 1927	Ophidia	70–90	1.3			well past excretory vessels
figurata Meggitt 1927	Insectivora	11–16	1.0	250		to excretory vessels
fusca Meggitt 1927	Ophidia	30–40	0.54			passing excretory vessels
herpestis Kofend 1917	Carnivora Insectivora	60	3.0	290		to excretory vessels
ichneumontis Baer 1924	Carnivora	100	4.0			to nerve
incisa (Marotel 1899)	Carnivora	50–150	1.3	270	80	
khalili Hamid 1932	Ophidia		1.9	570	200–220	to excretory vessels
lagrangei Joyeux and Houdemer 1927	Lacertilia	60	2.5	400		well past excretory vessels
lemuris (Beddard 1916)	Prosimeae	75	4.0	1,000		to excretory vessels
megastoma (Diesing 1850)	Primates	650	5.0	660		not reaching excretory vessels
parva Baylis 1929	Lacertilia	8–11	0.3–0.5	220–240	100–110	one-third proglottis
parva (Sandground 1926)	Carnivora					
rostellata Zschokke 1905	Ophidia	60–130	2–3	800–1,000	500	
surinamensis (Cohn 1902)	Edentata Marsupialia	160	3.6	600–900		to or past excretory vessels
symmetrica (Baylis 1927)	Rodentia	130	2.0	420	150	
tetragonocephala (Bremser 1856)	Edentata	150–400	2.4–5	1,500		
theileri Fuhrmann 1924	Lacertilia	4–8	0.85	300–400	140	to or past excretory vessels
trachysauri (MacCallum 1921)	Lacertilia	50	1.0	330	150	
tuberculata (Rudolphi 1819)	Lacertilia Ophidia	200	2.0	162–320	110	well past excretory vessels
zonuri Baylis 1919	Lacertilia	80–102	3.0	900–1,000	500	not to excretory vessels
americana n. sp.	Ophidia		0.6–0.8			well past excretory vessels
osheroffi n. sp.	Ophidia	60–205	1–1.4	370–400		to excretory vessels

TABLE I.—(*Continued*)

Length of cirrus-sac (in μ)	Number of testes	Arrangement of testes	Receptaculum seminis	Diameter of egg (in μ)	Diameter of onchosphere (in μ)
150	39–40	posterior to ovary	present	60	37
250	50	posterior to ovary	absent		
950	70–80	anterior, lateral and posterior to ovary			
111	22–24	posterior to ovary			
90	5–8	posterior to ovary		21	
150	40–50	posterior to ovary	absent	46	21–27
220	60	posterior to ovary			
83–150	20–30	posterior to ovary	? present	27–30	13
80	80–90	? mostly not posterior to ovary	present	52	20
150	20	anterior, lateral and posterior to ovary			
100	30–50	posterior to ovary	absent		
120–160	35–36	posterior and lateral to ovary			
86–108	24–33	posterior and lateral to ovary			
70–100	30–38	posterior to ovary			
220	50–60	posterior and lateral to ovary		57 × 32	
200	90–100	posterior and lateral to ovary passing excretory vessels	absent	46	30
100–130	23–30	posterior and lateral to ovary		30	23
286–318	44–61	posterior and lateral to ovary		70–80	40–48
250	50–60	posterior to ovary	present	50	18–20
	76	lateral and posterior to ovary	present		
100–150	160–200	exterior, lateral and posterior to ovary		50	
125	14–16	posterior and lateral to ovary		35–40	20–25
270–300	86–100	posterior and lateral to ovary			
340–450	100	posterior and lateral to ovary		50	
125	50–75	posterior and lateral		52–62	15
160	70–80	posterior to ovary	absent		
120	26–30	in two groups posterior to ovary	present	60	30–40
180	60	in two groups posterior to ovary	? absent		
260–300	20–40	posterior to ovary	absent	44	28–30
250	60–90	anterior, lateral and posterior to ovary	absent		
156–240	41–64	posterior and lateral to ovary	absent		
132–240	59–76	posterior and lateral to ovary	absent	32–60	22–42

1927 differs from *O. ameivae* (Beddard 1914) in the slightly larger number of the testes, the presence of additional lateral longitudinal excretory vessels, and the lesser width of the strobilus and should therefore become a synonym: *O. agamae* Baylis 1919 from *O. ameivae* in the slightly larger size of the strobilus and the slightly smaller size of the cirrus-sac and should become a synonym: *O. figurata* Meggitt, 1927 from *O. incisa* (Marotel, 1899) in the smaller length and slightly smaller size of the cirrus-sac and should become a synonym: *O. amphisbeteta* Meggitt 1924 from *O. erinacei* Meggitt 1920 in the lesser width and fewer testes and should become a synonym. The remaining species are less clearly defined and grade into one another. *O. parva** Baylis 1919 can be distinguished by its fewer testes, their arrangement, and the size of the cirrus-sac, *O. zonuri* Baylis 1919 by the anterior extension of the numerous testes and the small size of the cirrus-sac relative to the width of the proglottis, *O. cryptobothria* (Linstow 1904) by the small size of the cirrus-sac, the large number of testes and their arrangement, *O. tuberculata* (Rudolphi 1819) by the extent of the cirrus-sac, its large size, and the number of testes. Since it is improbable that the same species can parasitise both a reptile and a mammal, the remaining species may be divided into two groups. From those inhabiting reptiles, *O. rostellata* Zschokke 1905 may be distinguished by the large size of the cirrus-sac and the large number of testes, *O. fusca* Meggitt 1927, being separated from *O. crassiceps* Baylis 1920 only by the genital ducts in the latter species passing ventrally to the nerve, a character of dubious importance, becomes a synonym: the other species, being well differentiated from one another, are valid. Of those inhabiting mammals, *O. symmetrica* (Baylis 1927) should be considered a synonym of *O. lemuris* (Beddard 1916), the remaining species being well separated. A description of *O. parva* (Sandground 1926) not being available in Rangoon, that form is not included in the above comparison. The following list gives the names of the species of this genus in the light of the above remarks.

O. ameivae (Beddard 1914)

SYNONOMY.—*Linstowia ameivae: Oochoristica agamae* Baylis 1919: *O. brasiliensis* Fuhrmann 1927: *O. breslaui* Fuhrmann 1927.

HOSTS. —Lacertilia—**Agama sanguinolenta, A.* sp., *Hemidactylus brookii* Gray 1845, *Ameiva ameiva* (L. 1758), *A.* sp., *Tropidurus hispidus* (Spix 1825).

Ophidia—*Cerastes vipera* (L. 1758), *Dendropus* sp.

O. amphisbaenae (Rudolphi 1819)

SYNONOMY.—*Taenia amphisbaenae.*

HOSTS. —*Amphisbaena alba* (L. 1758) (Lacertilia).

* The specific name *parva* is preoccupied by *O. parva* (Sandground 1926) and the species of Baylis should thus be renamed.

Names marked * have not been checked and may be synonyms.

O. bivittata Janicki 1904

HOSTS. —*Didelphis marmosae, D. marsupialis* L. 1758, *D. paraguayensis* Oken 1816, *Marmosa elegans* (Waterhouse 1839), *M. murina* (L. 1758), *Metachirus nudicaudatus* (Geoffroy 1803), *M. opossum* (L. 1758), *Philander philander* (L. 1758) (Marsupialia).

O. crassiceps Baylis 1920

SYNONOMY.—*O. fusca* Meggitt 1927, *O. sigmoides* Moghe 1926.
HOSTS. —Lacertilia—*Calotes versicolor* (Daudin 1802), *Gongylus* sp. *Agama stellio* (L. 1758).
Ophidia—*Eryx jaculus* (L. 1758), **Psammophis subtaeniatus* Peters 1882.

O. cryptobothria (Linstow 1904)

SYNONOMY.—*Ichthyotaenia cryptobothrium.*
HOSTS. —*Chrysopelea ornata* (Shaw 1802).

O. didelphydis (Rudolphi 1819)

SYNONOMY.—*Taenia didelphydis.*
HOSTS. —*Marmosa elegans* (Waterhouse 1839), *M. murina* (L. 1758) (Marsupialia).

O. erinacei Meggitt 1920

SYNONOMY.—*O. amphisbeteta* Meggitt 1924.
HOSTS. —Carnivora—*Herpestes auropunctatus* (Hodgson 1836).
Insectivora—*Erinaceus algirus* Duvernoy & Lereboullet 1840, *E.* sp., *Macrocoelides rozeti* Smith 1839.
Rodentia—*Meriones shawi* (Duvernoy 1834), *Mus musculus* (L. 1758).

O. fibrata Meggitt 1927

HOSTS. —Ophidia—*Boiga cyanea* (Dumeril & Bibron 1854), *B. multimaculata* (Boie 1827).

O. herpestis Kofend 1917

HOSTS. —Carnivora—*Herpestes sanguineus* Rüpp. 1835.
Insectivora—*Atelerix spiculus* (Thomas & Wroughton 1907), *A. spinifex* Thomas 1918.

O. ichneumontis Baer 1924

HOSTS. —Carnivora—Foetorius sp., *Galerella gracilis* (Rüppell 1835).

O. incisa (Marotel 1899)

SYNONOMY.—*O. figurata* Meggitt 1927, *Taenia incisa.*
HOSTS. —Carnivora—*Meles meles* (L. 1758).
Insectivora—*Crocidura caerulea* (Kerr 1792).

O. khalili Hamid 1932

HOSTS. —Ophidia—*Psammophis schokari* (Forskal 1775).

O. lagrangei Joyeux & Houdemer 1927

HOSTS. —Lacertilia—*Leiolepis belliana* (Gray 1827).

O. lemuris (Beddard 1916)

SYNONOMY.—*Linstowia lemuris.*
HOSTS. —Prosimiae—*Nycticebus coucang* (Boddeart 1785).

O. megastoma (Diesing 1850)

SYNONOMY.—*Taenia megastoma.*
HOSTS. —Primates—*Alouatta belzebul* (L. 1766), *A. caraya* (Humbolt 1809), *Ateles belzebuth* Geoffory 1806, *Brachyteles arachnoïdes* Geoffroy 1806), *Callicebus amictus* (Geoffroy 1812), *C. calligatus* (Wagner 1842), *C. personatus* (Geoffroy 1812), *C. torquatus* (Hoffmann 1842), *Cebus apella* (L. 1758), *C. cirrifer* (Geoffroy 1812), *C. fatuellus* (L. 1766), *C. macrocephalus* Spix 1823, *C. variegatus* Geoffroy 1812, *Mico melanurus* (Geoffroy 1812), *Mystax ursulus* (Humbolt 1811), *Seniocebus bicolor* (Spix 1823), monki-monki.

O. parva Baylis 1929

HOSTS. —Lacertilia—**Lygosoma chalcides.*

O. parva (Sandground 1926)

SYNONOMY.—*Atriotaenia parva.*
HOSTS. —Carnivora—*Nasua narica* (L. 1766).

O. rostellata Zschokke 1905

HOSTS. —Ophidia—*Cerastes cerastes* (L. 1758), *Coluber jugularis* L. 1758.

O. surinamensis (Cohn 1902)

SYNONOMY.—*Taenia acephala* Creplin, *T. surinamensis.*
HOSTS. —Edentata—*Dasypus* sp., *Priodontes giganteus* (Geoffroy 1803), *Dasypus novemcinctus* L. 1758.
Marsupialia—*Didelphis marsupialis* L. 1758.

O. tetragonocephala (Bremser 1856)

SYNONOMY.—*Oochoristica wageneri* Janicki 1904, *Taenia tetragonocephala.*
HOSTS. —Edentata—*Myrmecophaga tridactyla* L. 1758, *Tamadua tetradactyla* (L. 1758).

O. trachysuri (MacCallum 1921)

SYNONOMY.—*Oochoristica theileri* Fuhrmann 1924, *Taenia trachysuri.*
HOSTS. —Lacertilia—*Agama hispida* (L. 1758), *Trachydosaurus rugosus* Gray 1825.

O. tuberculata (Rudolphi 1819)

SYNONOMY.—*Oochoristica pseudopodis* (Krabbe 1879), *O. rotundata* (Molin 1859), *O. truncata* (Krabbe 1879), *Taenia crenata* Krabbe 1879, *T. pseudopodis, T. rotundata, T. truncata, T. tuberculata.*
HOSTS. —Lacertilia.—*Acanthodactylus pardalis* (Lichtenstein 1823), *Agama agama* (L. 1758), *Chalcides ocellatus* (Forskal 1775), *Eumeces schneiderii* (Daudin 1802), *Lacerta agilis* L. 1758, *L. lepida* Daudin 1802, *L. muralis* (Laurenti 1768), *L. viridis* (Laurenti 1768), *L.* sp., *Ophiosaurus apodus* (Pallas 1775), **Ophisaurus pallasii, Uromastix acanthinurus* Bell 1825, *Varanus griseus* (Daudin 1803).

Ophidia—**Chrysolamprus ocellatus, Eryx jaculus* (L. 1758), *Psammophis sibilans* (L. 1758).
Isopoda—**Oniscus murarius.*

O. zonuri Baylis 1919

HOSTS. —**Zonurus tropidostomum.*

O. sp. Beddard 1911

HOSTS. —Edentata—*Tamandua tetradactyla* (L. 1758).

O. sp. Douthitt 1915

HOSTS. —Rodentia—*Geomys bursarius* (Shaw 1800).

O. sp. Janicki 1906

SYNONOMY.—*Taenia tetragonocephala* Parona 1901.
HOSTS. —Marsupialia—*Dasypus* sp.

O. sp. Joyeux 1923

HOSTS. —Ophidia—*Cerastes cerastes* (L. 1758).

O. sp. Joyeux 1923

HOSTS. —Lacertilia—*Varanus griseus* (Daudin 1803).

O. sp. Meggitt 1927

HOSTS. —Lacertilia—*Agama.* sp.

O. sp. Meggitt 1927

HOSTS. —*Agama stellio* (L. 1758).

O. sp. Zschokke 1905

HOSTS. —*Agama stellio* (L. 1758).

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OBSERVATIONS ON THE BLOW-FLY *LUCILIA SERICATA* MEIG[1]

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This paper is a report of progress after a year's work on the blow-fly *Lucilia sericata*. There is wide-spread interest in this species at present due to its successful use in surgery.

Adult *L. sericata* require detailed attention when kept in cages at a constant temperature of 25° C. An abundance of fresh water is essential at all times, and either sugar or meat should be kept in the cages for food. Granulated sugar was suggested by Dr. D. F. Miller and found to be preferable to syrup or honey. The flies thrived on the sugar and ground fresh beef diet. Occasionally yeast and banana were placed in the cages and were fed upon profusely. The yeast did not appear attractive until it had been in the cage a day or two. Liver was at times added to the beef, but the flies seemed to do just as well with the diet of sugar and ground beef alone.

The ground beef is placed in petri dishes and inserted into the cage. When it is placed in a cage for the first time, the flies swarm over the meat almost immediately and begin feeding. Three days after feeding on meat oviposition is begun. The flies lay eggs beneath the outer margins of the meat, usually tucked well under the meat so that it is often difficult to see the eggs without inverting the dish and examining the lower surface. However, when the lower edges of the meat appear to be thoroughly covered with eggs, oviposition will be noticed on the upper surface, especially in crevices.

An interesting observation was made in this connection which is an exception to the rule of placing the eggs beneath the meat. Adults which were ovipositing freely in a cage were placed in a photographic dark room at the same temperature, 25° C. Meat was placed in the cage, and the

[1] This work was carried out at the Zoological Laboratory, Duke University, 1931–32. I wish to express my sincere appreciation to Dr. A. S. Pearse for helpful suggestions at all times. Dr. F. G. Hall has kindly aided me with apparatus. Dr. W. A. Perlzweig of the medical faculty has generously helped me with numerous problems. Dr. L. A. Bigelow of the Chemistry Department has kindly aided me with suggestions. Dr. D. F. Miller of Ohio State University, Dr. Walter R. Sweadner of the University of Pittsburgh, Dr. F. F. Murdock of the U. S. Naval Medical School, Drs. William Robinson and G. F. White of the U. S. Bureau of Entomology, Dr. Edward F. Roberts of the Lederle Laboratories, Dr. George E. Bennett of Johns Hopkins University, Mrs. Elizabeth K. Elgin of the Children's Hospital School, Baltimore, have all sent me stock material and much valuable information which is greatly appreciated. Dr. Z. P. Metcalf, professor of Zoology, N. C. State College, read the manuscript and offered many helpful suggestions.

door quickly closed, leaving the cage in absolute darkness. Twenty-four hours later the dark room was quickly entered and the meat immediately removed for examination. Eggs were noticed profusely scattered over the surface of the meat, in sharp contrast to the usual careful oviposition beneath the edges. Only a few scattered eggs were noticed on the lower surface. This test was repeated several times leaving the cages in the dark room one hour before placing meat in the cages. Upper surface oviposition was noticed each time with only a few scattered eggs beneath.

Flies will live through an extended period in cold storage with neither food or water. Larvae and pupae can be kept in storage in excellent condition. Dr. D. F. Miller sent me a supply of pupae which had been in storage for two weeks at 8° C. The adult emergence was excellent. A group of flies were removed from petri dishes immediately after emergence from the pupa cases and placed at once in storage (dark) at 2° C. without having had food or water. Twenty-five percent of these flies were alive when removed from storage sixteen days later. Sexes were approximately equally distributed. The surviving flies were removed to normal conditions and given food and water. Longevity and oviposition were normal.

Flies which received plenty of food and water were kept for 30 days at 10° C. and were in excellent condition when removed, ovipositing normally.

One hundred eggs were placed on each of the following endocrine glands:[2] adrenals, ovaries, pancreas, thymus, and thyroid. Ground beef was used as a check. Sufficient material was placed in the jars to complete the larval development. This series of jars were placed in storage at 10° C. in order to reduce metabolism in an attempt to observe any differential in comparative development of the larvae. The eggs readily hatched at 10° C. and larval development was begun. Larvae from all endocrine glands and the beef began pupation at approximately the same time. However, a differential was noticed in the length of the pupal period. Flies from the thyroid emerged first in large numbers. The following day four flies from the ovaries emerged. A week later adults from the beef, adrenals, and thymus began emerging at the same time. The flies from the ovaries emerged slowly and completed emergence along with those from the beef, adrenal, and thymus. The thyroid flies had almost completed emergence before the beef, adrenals, and thymus flies had begun emergence. Larvae reared on thymus were unusually large in size and strikingly white in color, in contrast to the usual yellowish tinge.[3]

[2] Suggested by Dr. Bert Cunningham. Dr. C. D. Grinnells (N. C. Experiment Station) kindly furnished me with endocrine glands from cows.

[3] This work should be carried out on a large scale and repeated many times before definite conclusions can be drawn.

Wild flies readily oviposited through the cheese-cloth coverings of jars containing decaying meat, although the covers were six inches or more from the meat. Eggs were placed around the edges of muslin covers tucked between the rubber bands and the flange at the top of the jar by wild flies; however, such oviposition could never be induced in the cages of *Lucilia sericata* even though the cheese-cloth covering was only a fraction of an inch from the meat.

An attempt was made to obtain oviposition[4] by using a wide variety of organic compounds, clays and foods. In addition to meat, eggs were obtained on ground *L. sericata* larvae, yeast cake, banana, and beef extract agar. Oviposition was obtained on ground beef while the flies were in storage at a temperature of 10° C. No oviposition was obtained when the temperature was reduced to 2° C. When *L. sericata* flies were given a choice between fresh and rancid meat for egg laying, a marked preference was shown for the rancid meat.

L. sericata eggs hatch readily in distilled water at room temperature. Eggs were placed in a vial containing undiluted Hexylresorcinol–(St 37), shaken well for separation, and left for one hour at room temperature. All eggs hatched upon removal and the larvae appeared normal.

L. sericata larvae are very sensitive to moisture conditions. Larvae will feed normally in a jar of fresh ground meat, but, if too much moisture is formed by the decomposition of the meat, the young maggots will attempt to migrate long before time of pupation. Several hundred grown larvae were placed in a finger-bowl and left uncovered. Escape was not attempted; however, immediately upon the addition of a small amount of water, the larvae crawled over the edge of the bowl *en masse*. Twenty-five full grown larvae were placed in each of six finger-bowls; each bowl containing 100 cc. of distilled water. (When the larvae were dropped into a liquid in which they became completely immersed, there was no attempt at escape.) At twenty-four hour intervals, the larvae from one

TABLE 1.—*Immersion Tests in Distilled Water; 25 Larvae to Each Finger Bowl; Temperature 25° C; Shaded Light; No Agitation**

	Alive	Dead
Period of immersion (complete)— 24 hrs.	25	0
" " " " — 48 "	25	0
" " " " — 72 "	20	5
" " " " — 96 "	3	22
" " " " —120 "	0	25
" " " " —144 "	0	25

* No adult emergence records kept.

[4] In all tests for oviposition, egg laying was first obtained on ground beef to insure ability to oviposit.

finger bowl were carefully removed and placed on dry filter paper in a petri dish. Twenty-four hours later the removed larvae were examined in a bright light to determine whether dead or alive. Larvae often appear dead for several hours after being removed from any liquid. Table 1 gives the results of these immersion tests.

When larvae were immersed in various liquids without agitation, the results noted in Table 2 were obtained.

TABLE 2.—*Twenty-Four Hour Complete Immersion at 25° C. Twenty-Five Mature Larvae to Each Finger Bowl; No Agitation**

	Alive	Dead
Acid, Carbolic—2%	0	25
" Lactic—1%	21	4
" Oxalic—1%	8	17
" Picric—1%	0	25
Copper Sulphate—3%	22	3
Hexylresorcinol-St 37—2%	18	7
" 10%	4	21
Magnesium Sulphate—2%	25	0
" " —5%	10	15
" " —10%	8	17
" " —15%	1	24
" " —25%	1	24
Mercuric Chloride—1%	0	25
Red Arrow (Pyrethrum) 5%	0	25
Ringer's solution (undiluted)	13	12
Sunoco Spray Oil—3%	19	6

* All dilutions made with distilled water; examinations made 24 hours after removal from liquid; no adult emergence records kept.

The next table (Table III) shows the results obtained when tubes containing 25 mature larvae were three-fourths filled with the specified liquids and at once placed in an electric agitator, which threw the larvae and liquid successively from one end of the tube to the other. Agitation was continued for 24 hours. At the end of 12 hours a stop of 5 minutes was made and the stoppers were removed for an instant. The temperature was 25° C.

Acid dilutions according to pH would have been desirable; however it is hoped that the preliminary results recorded will point the way for future work.

SUMMARY

In North Carolina oviposition of *L. sericata* under natural conditions (exposing meat) was obtained every month of the year. Eggs are usually laid beneath the outer edges of the meat; however, in a dark room, most of the oviposition was on the upper surface. Adults can be kept for an extended period at 10° C. Oviposition, hatching, larval development, pupation, and adult emergence will take place at 10° C. In addition to meat, eggs were obtained on yeast, banana, beef extract agar and ground up blow-fly larvae. When given choice of fresh and rancid meat, adults show marked preference for the latter for oviposition. Larvae fed readily upon cow endocrines. In the one series carried out, thyroid

TABLE 3

	Alive	Dead	Adult Emergence
Acid, Acetic—2%	1	24	*
" , Boric—2%	25	0	15
" , Citric—2%	13	12	*
" , " —4%	1	24	*
" , Formic—2%	5	20	*
" , Hydrochloric—2%	11	14	*
" , Lactic—2%	1	24	*
" , " —10%	0	25	—
" , Nitric—2%	0	25	—
" , Oxalic—2%	0	25	—
" , Sulphuric—2%	2	23	*
" , Tannic—2%	23	2	17
" , Tartaric—2%	24	1	14
Alcohol—50%	0	25	—
Copper Sulphate—2%	8	17	2
" " —5%	15	10	7
Ether—5%	22	3	1
Mercuric Chloride—2%	0	25	—
Nicotine—5%	8	17	*
" —10%	23	2	6
" Sulphate—2%	11	14	*
Potassium Dichromate—2%	13	12	0
" Hydroxide—2%	10	15	*
Sodium Chloride—25%	5	20	*
" " —50%	0	25	—
" Hydroxide—2%	0	25	—
Water (Distilled)	24	1	14
" "	23	2	14
" "	25	0	20
" (Tap)	11	14	*
" "	15	10	*

* No adult emergence records kept. All dilutions made with distilled water. Examinations made 24 hours after removal from liquid.

seemed to shorten the pupal period. Larvae are very sensitive to excess moisture. Full grown larvae display a remarkable ability of survival after 24 hour immersions in various liquids.

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RESEARCH NOTES

A CHECK-LIST OF THE COCCIDIA OF THE GENUS *ISOSPORA*

The genus *Isospora* is to be distinguished from all other members of the family Eimeriidae by the fact that the protoplasmic mass or zygote inside the oocyst develops into two spores, each of which in turn comes to hold four sporozoites. In the preparation of this check-list, all forms conforming to this definition were regarded as belonging to the genus *Isospora*. The list is intended to show the specific name, the donor of the specific name, the date of the naming, the range in size (R) of the oocyst or the mean size (M) of the oocyst, and the known hosts—both the scientific and the common names. The writer invites correspondence regarding any possible omissions or errors.

Genus *Isospora* Schneider, 1881

Synonyms: *Psorospermium Rivolta*, 1878, *partim*; *Diplospora* Labbé, 1893; *Hyaloklossia* Labbé, 1898, *partim*; *Lucetina* Henry and Leblois, 1925.

Isospora ameivae Carini, 1931. M 19×16. In *Ameiva ameiva*, lizard.

I. belli Wenyon, 1923. R 25–33×12.5–16.5. In *Homo sapiens*, man.

I. bigemina Stiles, 1891. R 10–16×7.5–10.0 (small form). R 18–20×14–16.0 (large form). In *Canis familiaris*, dog; *Felis domestica*, cat; and other carnivores.

I. bigemina var. *canivelocis* Weidman, 1915. R 25–30. In *Vulpes velox*, swift fox.

I. bigemina var. *canis* Railliet and Lucet, 1891. R 12–15×7–9. In *Canis familiaris*, dog.

I. bigemina var. *cati* Railliet and Lucet, 1891. R 8–10×7–9 (Sporocyst measurement). In *Felis domestica* cat.

I. bigemina var. *putorii* Railliet and Lucet, 1891. R 8–12×6–8 (Sporocyst measurement). In *Putorius foetidus* (=*Mustela putorius*), polecat.

I. buteonis Henry, 1932. R 16–19.2×12.8–16.0. In *Buteo borealis, B. swainsoni, Accipiter cooperii*, hawks; *Asio flammeus*, owl.

I. camillerii Hagenmüller, 1898. M 22.0×22.0. In *Gongylus ocellatus*, lizard.

I. canis (*Vide I. bigemina* var. *canis*).

I. canivelocis (*Vide I. bigemina* var. *canivelocis*).

I. cati Marotel, 1921, (homonym; *cf. I. bigemina* var. *cati*). In *Felis domestica*, cat.

I. communis passerum Sjöbring, 1897. In *Fringilla* and *Sylvia* species, *Emberiza citrinella, Motacilla alba, Luscinia phoenicurus, Muscicapa atricapilla, Picus viridis, Inyx torquilla, Cuculus canorus, Lanius collurio, Corvus cornix*—all passerine birds.

*I. coelopeltis** Galli-Valerio, 1926. M 10.5×6.0. In *Coelopeltis lacertina*, snake.

*I. crotali** Triffitt, 1925. M 10.0×10.8 (Sporocyst measurement). In *Crotalus confluentus*, snake.

* Genus.?

I. dirumpens Hoare, 1933. R 15.2×11.2–16.8×9.6. In *Bitis arietans*, puff-adder.

I. dutoiti Yakimoff, Matikaschwili and Rastegaïeff, 1933. R 10.5–12.5×9.5–11. M 11.5×9.6. In *Canis aureus*, jackal.

I. felis Wenyon, 1923. R 39–48×26–37. In *Canis familiaris*, dog; *Felis domestica*, cat; and possibly certain other carnivores.

I. fragilis Leger, 1904. In *Vipera aspis*, viper.

*I. henryi** Yakimoff and Matikaschwili, 1932. R 37–42×30–35. In *Bubo bubo*, owl.

I. hominis Railliet and Lucet, 1891. In *Homo sapiens*, man.

I. hylae Mesnil, 1907. R 30–35×20–25. In *Hyla arborea*, tree frog.

I. lacazii Labbé, 1893. R (diameter) 23.0–25.0. In goldfinch, lark.

I. laidlawi Hoare, 1927. R 32–36.8×27.2–30.4. M 34×29. In *Putorius putorius* var. *furo,* ferret.

I. laverani Hagenmüller, 1898. M 12.0×10.0. In *Coelopeltis lacertina,* snake.

I. lieberkühni Labbé, 1894. Length 40 μ. In *Rana esculenta,* frog, adult and tadpole.

I. lyruri Galli-Valerio, 1931. M 15.0×15.0. In *Lyrurus tetrix,* grouse.

I. mesnili Sergent, 1902. M 30.0×30.0. In *Chamaeleo vulgaris,* chameleon.

I. mustelae Galli-Valerio, 1932. M 7.0×2.25. In *Mustela martes,* pine marten.

I. naiae Fantham, 1932. R 13.6×8.2–20.0×14.3. M 17×11. In *Naja flava,* snake.

I. neivai Pinto and Maciel, 1929. R 19–21×11–13. In *Bothrops jararaca* (= *Lachesis lanceolatus*), snake.

I. nucifragae Galli-Valerio, 1933. M 24.0×21.0. In *Nucifraga caryocatactes,* nutcracker.

I. passerum (*Vide I. communis passerum*).

I. putorii (*Vide bigemina* var. *putorii*).

I. rara Schneider, 1881 (type species). In "black slug" (*Limax cinereo-niger* or *L. griseus?*).

I. rastegaiev Yakimoff and Matikaschwili, 1933. R 16.5–21×15.4–20. M 19.1×17.4. In *Erinaceus europaeus,* hedgehog.

I. rivolta Grassi, 1879. R 20–25×15–20. In *Canis familiaris,* dog; *Felis domestica,* cat.

I. rivoltae Labbé, 1893 (homonym). R (diameter) 16.0–18.0. In chaffinch, shrike, titmouse.

I. sp. Grassi, 1881. M 7.0×4.5 (Sporocyst measurement). In *Coronella austriaca,* snake.

I. sp. Kotlan and Pospech, 1933. R 26–34×19–27. In *Meles taxus,* badger.

I. sp. Wenyon, 1926. In *Bufo vulgaris,* English toad.

I. sp. Wenyon, 1926. In *Agama colonorum,* lizard.

I. sp. Wenyon, 1936 (Reporting Adler's observation). In *Varanus griseus,* lizard.

I. suis Biester, 1934. M 22.5×19.4. In *Sus scrofa,* pig.

I. theileri Yakimoff and Lewkowitsh, 1932. M 21.2×17.1–18.0. In *Canis aureus,* jackal.

I. viverrae Adler, 1924. R 19.0–27.5×15.2–24.7. Common size 22.8×19. In *Viverra civetta,* civet cat.

I. vulpis Galli-Valerio, 1931. M 25.0×24.0. In *Vulpes vulgaris,* red fox.

I. wladimirovi Yakimoff, 1930. R 18–25×15.5–21.22. M 21.44×17.66. In *Hyla arborea,* tree frog.—Elery R. Becker, *Iowa State College.*

OBSERVATIONS ON FIRST-STAGE LARVAE OF *GASTEROPHILUS INTESTINALIS* IN TONGUES OF HORSES IN CENTRAL IOWA DURING DECEMBER, 1932, and JANUARY, 1933

Treating horses for bots (*Gasterophilus* spp.) has been given considerable attention in many states in recent years. This has led to more interest in the destruction of *Gasterophilus intestinalis* eggs on the host, before internal treatment, for it is well known that many eggs of this species remain viable for some time after fly activity has ceased. The studies of Dinulescu (Ann. Sc. Nat. Zool. (10), 15:1–183, 1932) and Wehr (Jour. Parasit., 19:252, 1933) have shown that the first larval period is spent in the mucous layers of the tongue. According to Wehr this period in the tongue lasts from 21 to 28 days. This habit of remaining in the tongue for a definite period sugested the problem of making post-mortem examinations of tongues at regular intervals to determine the extent, and during what period after oviposition has ceased, the stomach of the horse may be reinfested from viable larvae in the eggs and from larvae already in the tongue.

This point was determined, for Central Iowa, for the season December, 1932, and January, 1933, by post-mortem examination of horses' tongues at various intervals.

All examinations of tongues were made macroscopically. One familiar with the burrowing habits and lesions caused by the first-stage larvae can recover a high percentage, if not all, of the larvae present in the tongue.

The number of tongues examined each period and the corresponding number of larvae found are listed in the table. For convenience, the numbers examined are grouped in definite periods in December, 1932, and in January, 1933. The tongues were obtained from horse carcasses, brought to a carcass disposal plant, and examined immediately upon arrival. Exact dates of death of the horses were unascertainable but it is probable that death had occurred in each case within two or three days previous to arrival at the plant.

TABLE 1.—*Number of Tongues Examined and Larvae Recovered During Each Period for December and January*

Dates 1932–1933	Number of Tongues Examined	Number of larvae found		Average number of larvae per tongue
		small	large	
Dec. 1–7	5	7	45	10.40
Dec. 8–14	8	46	162	26.00
Dec. 15–21	5		23	4.60
Dec. 22–31	2		7	3.50
Jan. 1–7	9	2	27	3.20
Jan. 8–14	9		14	1.55
Jan. 15–21	9		1	0.11

By noting the variations and averages it can be seen that a large number of larvae were present in the tongue as late as December 15. After this date there was a rapid decline in the average number, yet certain individual tongues had a comparatively large number as late as January 7. Since only one larva was found in nine tongues during the period January 15–21, no further observations were made at a later date.

In the table the larvae are arbitrarily grouped into large and small classes in order to indicate the possible time that they were taken into the mouth. Since very few small larvae were found after December 8–14, indications are that the number ingested was negligible after December 14. This is borne out later by the fact that examinations of tongues thirty days from this date produced very few larvae.

Present studies indicate that for central Iowa it is essential that eggs be destroyed on horses if they are to be treated with carbon disulphide before January 15, as observations indicate that the number of viable eggs on the host up to December 15 is sufficient to produce a reinfestation.

It appears, though based on only one season's observations, that for central Iowa the destruction of eggs on the host is not necessary if larvifuge treatments are to be administered after January 15, for very few larvae were recovered after this date, and tongues examined for four weeks before this date produced mostly larvae well advanced in the first instar. Further studies will be necessary to determine if variations, in the last date that larvae may be found in the tongue, occur from year to year.—E. F. KNIPLING, *Division of Insects Affecting Man and Animals, U. S. Bureau of Entomology.*

PRELIMINARY STUDIES ON THE PRODUCTION OF BIRD MALARIA INFECTIONS BY THE INJECTION OF SPOROZOITES

The following note comprises a brief summary of the studies that were undertaken with a view of determining the following points:

1. The minimum number of sporozoites that can bring about an infection in birds.

2. The duration of life of sporozoites *in vitro*.

3. Can sporozoites bring about superinfection in latent infections?

Canaries (*Serinus canarius*) were used as hosts and *Plasmodium cathemerium* Hartman, 1924, as the parasite; *Culex pipiens* Linnaeus bred out in the laboratory as the transmitting agent. The adult female mosquitoes were allowed to feed on heavily infected birds and were kept for 15 to 18 days thereafter under prevailing laboratory conditions. During this period they were fed on raisins and water. The mosquitoes were dissected fifteen days after feeding under conditions which were as nearly aseptic as possible. In positive cases where the sporozoites were seen actually wriggling in and around the salivary gland, a suspension of the gland was made in 1 cc. of sterile Locke's Solution. One one-hundredth of a cubic centimeter of this suspension was spread over 1 sq. cm. of a glass slide and stained with Giemsa. The number of sporozoites was counted in this film and from this the total number of sporozoites per cc. of the suspension was estimated.

Different quantities of this standardized suspension were inoculated intramuscularly into nine clean birds. It was observed that when more than 200 sporozoites were injected into the bird an infection resulted. Following injection of less than 200, no parasites were found in the peripheral circulation of birds. The uninfected birds when subsequently reinoculated with infected blood became positive.

The viability of sporozoites *in vitro* was tested after suspensions of sporozoites had been kept in the refrigerator (5° C.) for intervals of 1, 2, 6, 12, 24 and 48 hours. It was observed that the sporozoites inoculated after 1 or 2 hours refrigeration were able to bring about infections, whereas the organisms refrigerated for the longer periods of time failed to infect birds. Attempts to infect birds with the suspension of sporozoites incubated at 37° C. were not successful as all the birds died of bacteraemia before the presence of parasites could be detected.

It is well known that canaries which have once survived an infection with a particular strain or species of bird malaria cannot be reinfected with the same species as long as the original infection is subpatent. The virulence of some pathogenic protozoa has been enhanced by passage through the insect host and an attempt was made to determine whether or not the passage of *P. cathemerium* through the mosquito would increase its virulence to the extent of bringing about superinfection in "previously infected" or "subpatent" birds.

Ten birds known to have latent infections were inoculated with heavy doses of viable sporozoites. Daily examination of blood smears for one month after the injection of sporozoites did not reveal the presence of parasites in the peripheral circulation.

In summing up the results it may be stated that the preliminary studies with *P. cathemerium* discussed above seem to suggest that, although theoretically one viable sporozoite should be able to produce an infection in birds, large numbers of sporozoites injected intramuscularly were necessary. It should be emphasized that the conditions of this experiment did not reproduce the conditions of natural inoculation by the mosquito and that our conclusions are correspondingly limited.

Sporozoites of *P. cathemerium* can stand refrigeration at 5° C. for a period of two hours but not for six hours or longer. Passage of the parasite through its insect host did not seem to increase its virulence to the extent of producing superinfection in birds.—L. E. Rozeboom and K. S. Shah, *Johns Hopkins University, School of Hygiene and Public Health.*

PASSAGE OF *TRICHOMONAS HOMINIS* IN A VIABLE CONDITION THROUGH THE STOMACH AND SMALL INTESTINE OF A MONKEY

In 1931 in Panama 4 cc. of a diarrheic stool containing large numbers of *Trichomonas hominis* were injected through a stomach tube into the stomach of a brown howler monkey (*Alouatta palliata palliata* Gray). The monkey was killed one hour and 50 minutes later and the stomach and intestine examined immediately. Trichomonads were still present in the pyloric end of the stomach; most of them exhibited movements but only a few were swimming about. Many active trichomonads were observed in the small intestine 20 cm., and 32 cm. posterior to the stomach; a few were present at the end of the ileum, 50 cm. posterior to the stomach. Active trichomonads were found in the caecum but these may have been there before the animal was used for the experiment.

There is only one conceivable method for human trichomonads, which do not form cysts, to reach the large intestine, which is the primary site of infection, and that is by the passage of trophozoites ingested in food and drink through the stomach and small intestine. That this is possible in rats, guinea-pigs, and cats has been demonstrated by several investigators (Brumpt, Ann. Parasit., 3: 239, 1925; Hegner, Amer. Jour. Hyg., 4:143, 1924; Hegner, Amer. Jour. Hyg., 6:593, 1926; Wenrich and Yanoff, Amer. Jour. Hyg., 7:119, 1927). No one has hitherto demonstrated this in a large animal. The fact that the organisms can safely pass from the stomach to the large intestine of the monkey makes it seem more probable that they can undergo a similar journey in the human body and remain in a viable condition.—Robert Hegner, *Johns Hopkins University, School of Hygiene and Public Health.*

TRANSMISSION OF *ENDAMOEBA HISTOLYTICA* IN DRINKING WATER

The rôle played by drinking water in the transmission of *E. histolytica* is still in doubt, hence observations made during the summer of 1933 in Chiquimula, Guatemala, seem worth recording. Many of the missionaries living in the Friends Mission in Chiquimula have during the past decade come down with severe attacks of amoebic dysentery. About a year ago a well was drilled and a tank and oil-driven pump installed. Pipes were laid to 3 or 4 mission buildings and a pure water supply thus assured. Since then no cases of amoebic dysentery have developed. In June, 1933, I examined by smear one sample from each of 7 missionaries and 47 servants, working in the mission, and children, most of whom were living in the mission. No infections with *E. histolytica* were found among the 7 missionaries and only 5 among the 47 servants and children. No survey had been made before the pure water supply was installed, hence no comparison can be drawn between conditions before and after, but the data available indicate that the water supply in former years, which is known to have been much polluted, was responsible for the transmission of some of the infections.—Robert Hegner, *Johns Hopkins University, School of Hygiene and Public Health.*

PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The Helminthological Society of Washington was organized in Washington, D. C., U. S. A., by a group of scientists interested in the field of parasitology. In the meetings held the third Saturday of each month during the academic year members and guests present papers and research notes in the various fields of helminthology (including subjects dealing with free-living and plant nematodes), protozoology, entomology, and related subjects.

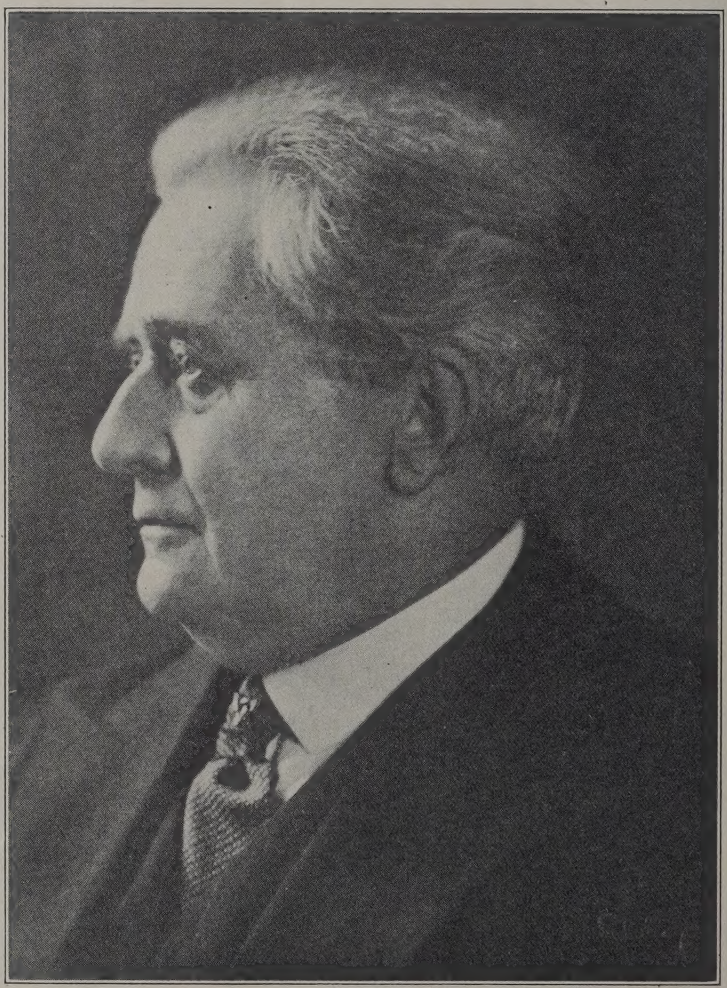
The proceedings of the first 15 meetings were published in *Science*. Those of the 16th to 20th meetings were published in the first number of the JOURNAL OF PARASITOLOGY in 1914 and have since been published in this journal up to the proceedings of the 155–156th meetings appearing in the December, 1933 issue (volume 20, number 2).

The proceedings have grown from a few notes representing the secretary's version of the meeting when the Society began to 92 separate research notes for the academic year 1932–33, occupying 61 pages in the JOURNAL OF PARASITOLOGY. The proceedings for the current academic year show evidence of being still larger and are yet to be published. Accordingly it seemed advisable to undertake the publication of the *Proceedings* of the Helminthology Society of Washington as a separate journal. Volume 1 will consist of two numbers, the first of which will appear in March, 1934 and will start with the proceedings of the 157th meeting, held October 21, 1933. While the journal is intended primarily as a medium for the publication of notes and short papers by members of the Society this journal offers quick publication of suitable papers by non-members if the author will contribute towards the cost of publication. The first Editorial Board consists of Jesse R. Christie, Editor, U. S. Bureau of Plant Industry, Washington; H. E. Ewing, U. S. Bureau of Entomology, Washington; G. F. Otto, Johns Hopkins University, Baltimore; E. W. Price, U. S. Bureau of Animal Industry, Washington, and L. A. Spindler, U. S. Bureau of Animal Industry, Washington. All business communications should be addressed to the Editor, Jesse R. Christie, while manuscripts for publication may be submitted to any member of the Editorial Board.

Subscription price per volume—Domestic, $1.00; Foreign, $1.25.

PUBLICATION OF RESEARCH NOTES IN THE JOURNAL OF PARASITOLOGY

The Editorial Committee of the JOURNAL OF PARASITOLOGY started in the September, 1933, number a section on "Research Notes." The purpose of this section is to make possible publication without undue delay of short notes on discoveries which do not lend themselves to publication as more extended articles. Abstracts or preliminary reports of researches the results of which are to be published later in more extended form will not be included, since the section will be reserved for the final publication of new discoveries. The text of a research note will be limited to one page of 8-point type or about 750 words. A limited amount of illustration and tabulated material can be added provided the author is willing to pay the cost. The purpose of the section is to give prompt publication to short articles. It is also hoped that the notes will add to the interest of the JOURNAL by giving greater diversity to individual numbers. This section is also serving another purpose in this time of publication difficulties, since in many cases authors have cut down articles which ordinarily would be published at much greater length to bring them into the size of a research note. We would like to call the attention of contributors who are planning to send in notes for this section to the form used in this number. Footnotes should be entirely avoided and necessary references to the literature should be given in the text at the place where the reference is made. The section on research notes is admittedly an experiment and the Editorial Committee would welcome any comments, criticisms, or suggestions in regard to it.



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